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# CONTENTS

## No. 1

BARRATT, J. O. WAKELIN ; and YORKE, WARRINGTON.

PAGE

An Investigation into the Mechanism of the Production of Blackwater. Plates

I, II, figures 1-82 . . . . . 1

## No. 2

KINGHORN, ALLAN ; and MONTGOMERY, R. EUSTACE.

On the Flagellates occurring in the Intestine of *Glossina palpalis*, and in the  
Intestine and Proboscis of *Glossina morsitans* . . . . . 259

KINGHORN, ALLAN ; and MONTGOMERY, R. EUSTACE.

Second Report on Human Trypanosomiasis in North-Eastern Rhodesia and  
Nyasaland . . . . . 277

MONTGOMERY, R. EUSTACE ; and KINGHORN, ALLAN.

A further Report on Trypanosomiasis of Domestic Stock in Northern Rhodesia  
(North-Eastern Rhodesia). Plates III, IV, and map . . . . . 311

## No. 3

CARDAMATIS, JOHN.

Sanitary Measures and Malaria Epidemics of Athens. Plates V-VII . . . . . 375

DAY, H. B. ; and FERGUSON, A. R.

An Account of a Form of Splenomegaly with Hepatic Cirrhosis, Endemic in  
Egypt. Plates VIII-XII . . . . . 379

BREINL, ANTON ; and NIERENSTEIN, M.

Bio-Chemical and Therapeutical Studies on Trypanosomiasis . . . . . 395

# CONTENTS

## No. 4

NEWSTEAD, ROBERT.

PAGE

- Reports of the Twenty-first Expedition of the Liverpool School of Tropical Medicine. Jamaica, 1908-1909; Section I—Medical and Economic Entomology. Part I—Ticks and other Blood-sucking Arthropoda. Plates XIII-XV, and 1 figure in text . . . . . 421

PROUT, W. T.

- Reports of the Twenty-first Expedition of the Liverpool School of Tropical Medicine. Jamaica, 1908-1909; Section II—Malaria. Plates XVI-XVIII 471

## No. 5

BREINL, ANTON; and HINDLE, EDWARD.

- Observations on the Life History of *Trypanosoma lewisi* in the rat louse (*Haematopinus spinulosus*). Plates XIX, XX . . . . . 553

YORKE, WARRINGTON.

- On the Variation of the Haemolytic Complement in Experimental Trypanosomiasis . . . . . 565

KENNAN, R. H.

- Acute Craw-Craw. Plates XXI, XXII . . . . . 581

WILLCOCKS, F. C.

- A Preliminary Note on the Prevalence of Mosquitoes in Cairo and its Environs 583

BOYCE, RUBERT; and LEWIS, F. C.

- The Effect of Mosquito Larvae upon Drinking Water . . . . . 591







## INDEX



# INDEX

INDEX OF AUTHORS .....	PAGE iii
GENERAL INDEX .....	iii
INDEX OF SPECIES NEW TO SCIENCE .....	viii

## INDEX OF AUTHORS

	PAGE		PAGE
Barratt, J. O. Wakelin; and Yorke, Warrington .....	I	Lewis, F. C.; and Boyce, Rubert...	591
Boyce, Rubert; and Lewis, F. C. ...	591	Montgomery, R. Eustace; and Kinghorn, Allan .....	259, 277, 311
Breinl, Anton; and Neirenstein, M.	395	Neirenstein, M.; and Breinl, Anton	395
Breinl, Anton; and Hindle, Edward	553	Newstead, Robert .....	421
Cardamatis, John .....	375	Prout, W. T. ....	471
Day, H. B.; and Ferguson, A. R....	379	Willcocks, F. C. ....	583
Ferguson, A. R.; and Day, H. B....	379	Yorke, Warrington; and Barratt, J. O. Wakelin .....	I
Hindle, Edward; and Breinl, Anton	553	Yorke, Warrington .....	565
Kennan, R. H. ....	581		
Kinghorn, Allan; and Montgomery, R. Eustace .....	259, 277, 311		

## GENERAL INDEX

	PAGE		PAGE
<i>Amblyomma cajanense</i> .....	440, 449	Birds, Food of Jamaican .....	449
„ <i>dissimile</i> .....	445	Blackbird, Parrot-billed, feeding on ticks	451
„ <i>maculatum</i> .....	443	Blackwater, An investigation into the mechanism of produc- tion of .....	I
<i>Anopheline</i> breeding pools. Plates V-VII .....	378	„ Blood cells, Haemolysis of red, by quinine bi- hydrochloride .....	18
<i>Anophelines</i> of Athens .....	375	„ Blood Cells and Quinine solution, Composition of mixture of .....	16
„ Bionomics of .....	471	„ Clinical records of .....	174
„ Egyptian; Notes on breeding places of ...	583	„ Haemoglobinaemia, Rela- tion of to haemoglo- binaemia in .....	69
„ List of Jamaican .....	486	„ Haemolysinaemia, Rela- tion of to the Haemo- globinuria in .....	62
<i>Anthrax lucifer</i> .....	469	„ Haemolysis of red blood cells by quinine .....	13
Antimony compounds, Effect of on Trypanosomes .....	412	„ Haemolysis of red blood cells by acid .....	13
Anti-malarial measures in Jamaica, etc.	472	„ Haemolysis of red blood cells by alkali .....	13
<i>Aponomma</i> sp. ....	447		
<i>Argas persicus</i> .....	434		
Athens, Sanitary measures and malaria epidemics of .....	375		
Atoxyl .....	396		
„ Derivatives of .....	403		
<i>Atylotus jamaicensis</i> , n. sp. ....	465		
Barratt and Yorke. The mechanism of production of Blackwater .....	I		
Birds destroying ticks .....	447		

	PAGE		PAGE
Blackwater, Haemolysis of red blood cells by urine .....	13	Breinl and Hindle. On the life-history of <i>Trypanosoma lewisi</i> in the rat louse .....	553
„ Hydrochloric acid, Action of, on red blood cells...	20	Breinl and Nierenstein. Bio-Chemical and therapeutical studies on Trypanosomiasis.....	395
„ Icterus and .....	170	<i>Bufo marinus</i> .....	455
„ Introduction to Report on .....	12	Bull Frog .....	455
„ Kidneys, the condition of in .....	166	Cairo, Breeding places of Anophelines in .....	583
„ List of contents of Report on .....	1	Cardamatis. On sanitary measures and malaria epidemics of Athens...	375
„ Mechanism of production of .....	136	Cattle, Distribution of, in North-Eastern Rhodesia .....	313
„ Prophylaxis and treatment of .....	171	„ washes and dips .....	458
„ Quinine, Action of on red blood cells affected with malarial parasites .....	49	<i>Cellia pharoensis</i> .....	584
„ Quinine, Action of on red cells during blackwater fever .....	44	<i>Chrysomyia macellaria</i> , Larvae of in man .....	462
„ Quinine, Action of on healthy red cells in the living body .....	43	<i>Chrysops costalis</i> .....	465
„ Quinine, Hydrochloric acid and sodium hydrate, Action of on red blood cells, compared .....	22	Cirrrosis, An account of a form of splenomegaly with hepatic, endemic in Egypt .....	379
„ Quinine in the form of alkaloid on red blood cells, Action of .....	19	Clinical records of blackwater fever...	174
„ Quinine in the alkaloidal state, Nature of the action of, on red blood cells .....	27	Craw-craw, Acute .....	581
„ Sodium hydrate, Action of on red blood cells...	21	<i>Crithidia</i> sp. in <i>Haematopinus spinulosus</i> .....	553
„ Summary of results .....	60	<i>Crotophaga ani</i> , feeding on ticks .....	451
„ Summary .....	92, 135, 165	<i>Culex fatigans</i> .....	585
„ Summary of report on...	169	<i>Culex</i> spp., effect of larvae of on drinking water .....	592
„ Tables, Index of .....	5	<i>Cyclops</i> sp., effect of on drinking water .....	593
„ Urine, the action of on red blood cells, and on dissolved haemoglobin .....	51	Day and Ferguson. A form of Splenomegaly with hepatic cirrhosis, endemic in Egypt .....	379
„ Urine, Mechanism of production of suppression of, in .....	94	<i>Dermacentor nitens</i> .....	439
Boyce and Lewis. The effect of mosquito larvae upon drinking water.....	591	<i>Dermatophilus (Sarcopsylla) penetrans</i> .....	469
		Dipterous flies infesting man .....	466
		Di-sodium Azobenzene 4-arsenate ...	404
		<i>Distomum hepaticum</i> .....	317
		<i>Dysdercus</i> sp. ....	451
		Egypt, A form of splenomegaly with hepatic cirrhosis, endemic in.....	379
		Egyptian <i>Anophelines</i> , breeding places of .....	583
		Ferguson and Day. A form of Splenomegaly with hepatic cirrhosis endemic in Egypt .....	379
		Flagellates occurring in the intestine of <i>Glossina palpalis</i> , and in the intestine and proboscis of <i>Glossina morsitans</i> .....	259



	PAGE
Formylo-Atoxyl, Effects of on Trypanosomes .....	404
Game, African. A list of the species examined for <i>trypanosomes</i> .....	371
Gland palpation and puncture .....	291
<i>Glossina fusca</i> in North-Eastern Rhodesia .....	284
"    in Nyasaland .....	284
<i>Glossina morsitans</i> , Flagellates occurring in the intestine and proboscis of .....	259
"    "    habits of .....	287
"    "    Incidence of intestinal contamination in .....	263
"    "    Incidence of infection in proboscis .....	264
"    "    in North-Eastern Rhodesia .....	283
"    "    in Nyasaland .....	283
"    "    in North-Western Rhodesia .....	284
"    "    in relation to the distribution of stock .....	313
"    "    in association with wild game .....	323
"    "    Morphology of Trypanosomes found in .....	265
"    "    Ratio of sexes in... ..	263
<i>Glossina palpalis</i> , Feeding experiments with .....	271
"    "    Flagellates occurring in the intestine of .....	259
"    "    Habits of .....	284
"    "    Incidence of intestinal contamination in .....	262
"    "    Incidence of infection in proboscis .....	264
"    "    in North-Eastern Rhodesia .....	281
"    "    in Nyasaland .....	283
"    "    in association with game .....	327
"    "    Morphology of forms of Trypanosomes found in ...	265

	PAGE
<i>Glossina palpalis</i> , on shores of Lake Tanganyika .....	317
"    "    physical characters of the country in which found .....	279
"    "    Ratio of sexes in... ..	262
<i>Glossina</i> spp. in relation to disease ...	328
"    Map showing distribution of .....	374
"    Trypanosomes, 'Wild' forms in .....	275
<i>Grabhamia willcocksii</i> .....	584
<i>Haematopinus spinulosus</i> , On the life-history of <i>Trypanosoma lewisi</i> , in ...	553
Haemoglobinaemia, Relation of to haemoglobinuria in blackwater fever .....	69
Haemolysinaemia, Relation of to the Haemoglobinuria in Blackwater fever .....	62
Herpetomonas in rat louse .....	555
Hindle and Breinl. On the life-history of <i>Trypanosoma lewisi</i> in the rat louse .....	553
Hippoboscidae of bats and birds... ..	467, 468
<i>Homophoeta equinoctialis</i> .....	451
Horn-fly in Jamaica .....	463
Hydrochloric acid, Action of on red blood cells .....	20
"    "    Sodium hydrate and Quinine, Action of on red blood cells .....	22
Icterus and Blackwater fever.....	170
<i>Ixodes bovis</i> . (See <i>Margaropus annulatus</i> ) .....	
" <i>ricinus</i> , Reputed occurrence of in Jamaica.....	422
" <i>scapulatus</i> . (See <i>Amblyomma cajanense</i> ) .....	
Ixodoidea in Jamaica .....	421
Jamaica, Ixodoidea of .....	421
"    Report on Malaria in .....	471
"    Ticks and other blood-sucking Arthropoda in .....	421
Kennan. Acute Craw-craw .....	581
Kharsin.....	406
Kidneys, the condition of in blackwater fever .....	166
Kinghorn and Montgomery. On Trypanosomiasis of domestic stock in Northern Rhodesia .....	311



	PAGE		PAGE
Kinghorn and Montgomery. On the flagellates occurring in the intestine of <i>Glossina palpalis</i> .....	259	<i>Pyretophorus cleopatrae</i> .....	586
Lewis and Boyce. The effect of mosquito larvae upon drinking water.....	591	" <i>superpictus</i> .....	375
<i>Libellulidae</i> , natural enemies of mosquito larvae .....	588	Quinine, Action of on red cells during blackwater fever .....	44
Lizards, Food of .....	454	"    Action of on red blood cells affected with malarial parasites .....	49
Louse, Rat. Observations on the life-history of <i>Trypanosoma lewisi</i> in the	553	"    Action of on healthy red cells in the living body .....	43
<i>Loxa flavicollis</i> .....	452	"    bihydrochloride in blood plasma .....	18
<i>Lyperosia irritans</i> in Jamaica .....	463	"    hydrochloric acid and sodium hydrate, Action of, on red blood cells .....	22
Malaria, Epidemics of, Athens, Sanitary measures and.....	375	"    in the alkaloidal state, Nature of the action of on red blood cells .....	27
"    General history of .....	478	"    in the form of alkaloid on red blood cells, Action of .....	19
"    in children at Athens, comparative tables .....	377	"    use of in acute attacks of malaria .....	533
"    in Jamaica, Report on.....	471	<i>Quiscalus crassirostris</i> , feeding on ticks	448
Malarial parasites, Action of on red blood cells, affected with malarial parasites .....	49	Redwater in piroplasmosis .....	165
<i>Margaropus annulatus</i> , var <i>australis</i> .....	435, 449, 452	<i>Rhipicephalus sanguineus</i> .....	437
Mosquito larvae, Effect of on drinking water .....	591	Rhodesia. On Trypanosomiasis of domestic stock in Northern .....	311
Mosquitos in Cairo and environs ...	583	Sanitary measures and malaria epidemics of Athens.....	375
"    Natural enemies of .....	588	Sleeping sickness. (See also Trypanosomiasis)	
Montgomery and Kinghorn. On the flagellates occurring in the intestine of <i>Glossina palpalis</i> ...	259	Sleeping sickness, Mode of introduction of.....	301
Montgomery and Kinghorn. On Trypanosomiasis of domestic stock in Northern Rhodesia .....	311	"    "    Occurrence of cases of .....	300
Myiasis in man .....	462	"    "    Prophylaxis .....	302
Newstead, Ticks and other blood-sucking <i>Arthropoda</i> in Jamaica .....	421	Sodium di-p-acetyl-amino-phenyl-arsenate .....	405
Niernstein and Breinl, Bio-chemical and therapeutical studies on Trypanosomiasis.....	395	"    di-p-amino-phenyl-arsenate .....	405
Notonectidae, Natural enemies of mosquito larvae .....	588	"    hydrate, Action of on red blood cells .....	21
<i>Ornithoetona erythrocephala</i> .....	467	"    "    Quinine and Hydrochloric acid, Action of on red blood cells .....	22
Orsudan, C. ....	406, 408	"    "    p-hydroxy-phenyl-arsenate .....	405
Piroplasmosis, Redwater in.....	165	Splenomegaly with hepatic cirrhosis, An account of a form of, endemic in Egypt .....	379
<i>Polistes crinita</i> in stomach of <i>Crotophaga ani</i> .....	451	Stock, Disease in .....	334
Prophylaxis and treatment of Blackwater fever .....	171		
Prout, Malaria in Jamaica.....	471		

	PAGE
<i>Stomoxys</i> and Tabanidae, said to be responsible for the transmission of Trypanosomiasis .....	331
<i>Stomoxys calcitrans</i> in Jamaica .....	464
Tabanidae and <i>Stomoxys</i> , reference to distribution of in Rhodesia .....	331
<i>Tabanus</i> sp., reference to .....	315
Tetra sodium phenazine 4-arsenate .....	405
<i>Theobaldia annulata</i> .....	592
<i>Theobaldinella spathipalpis</i> .....	574, 587
Ticks and other blood-sucking	
Arthropoda in Jamaica .....	421
Apparatus for spraying infested cattle .....	457
Dissemination of by various agencies .....	425
Effect of Rain or Water on .....	425
Life-cycles of .....	428
Longevity of without host ...	427
Natural enemies of .....	447
Prevailing conditions of infested pastures .....	426
Results obtained by burning infested pastures .....	455
Seasonal prevalence of in Jamaica .....	424
Structural characters of .....	430
Synopsis of Jamaica species ...	432
<i>Trichobius parasiticus</i> .....	468
<i>Trypanosoma cazalboui</i> .....	329
<i>dimorphon</i> .....	345, 373
<i>lewisi</i> , Observations on the life-history of in the rat louse <i>Haematopinus spinulosus</i> .....	553
Morphology of Balungu strain .....	351
Morphology of Chunga strain .....	361
Morphology of Kambola strain .....	348
Morphology of Ninamwenda strain .....	356
Morphology of Scotsdale strain .....	344
<i>nanum</i> .....	329
<i>vivax</i> .....	329, 373
spp., effect of colouring matters on .....	409
,, effect of drugs on .....	406

	PAGE
<i>Trypanosoma</i> spp., Reference to several African .....	347, 566
Trypanosomes, Effect of Atoxyl, etc.,	
on .....	396
in naturally infected pig .....	367
in naturally infected dogs .....	358
in naturally infected Ruminants .....	340
in African game .....	371
Methods of staining... ..	557
Morphology of cattle .....	373
Morphology of forms found in <i>Glossina palpalis</i> .....	265
Origin of 'Wild' forms in <i>Glossina</i> spp. ....	273
Permanent cure of in animals .....	418
Reappearance of after treatment with drugs .....	417
Resistance of to drugs .....	413
Transmission of cattle .....	374
Trypanosomiasis, Bio-Chemical and Therapeutical	
Studies on .....	395
Comparative value of experiments on .....	420
in North-Eastern Rhodesia and Nyasaland, Second report on Human .....	277
of domestic stock in Northern Rhodesia, A further report on .....	311
of domestic stock, supp. note .....	373
On the variation of the haemolytic complement in experimental .....	565
Prophylactic measure for... ..	337
Trypanred, Effect of on Trypanosomes .....	409
Tsetse flies. (See also <i>Glossina</i> )	

	PAGE		PAGE
Tsetse flies, distribution of in North-Eastern Rhodesia .....	281	Willcocks, Mosquitos in Cairo and its environs .....	583
„ Map showing distribution of in Rhodesia .....	374	Yorke and Barratt, The mechanism of production of Blackwater .....	1
Urine, Mechanism of production of suppression of in blackwater fever .....	94	Yorke, On the variation of the haemolytic complement in experimental Trypanosomiasis .....	565
„ The Action of on red blood cells and on dissolved haemoglobin .....	51		

#### INDEX OF SPECIES NEW TO SCIENCE

<i>Atylotus jamaicensis</i> , Newstead .....	465
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Miss Mary  
Patterson





# AN INVESTIGATION INTO THE MECHANISM OF PRODUCTION OF BLACKWATER

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## CONTENTS

INTRODUCTION, p. 12.

SECTION I. The haemolysis of red blood cells by quinine and also by acid, alkali and urine.

(a) Action of quinine, acid and alkali on healthy red blood cells : action of quinine bihydrochloride in varying concentrations on red blood cells, p. 13 ; action of quinine in the form of alkaloid on red blood cells, p. 19 ; action of hydrochloric acid on red blood cells, p. 20 ; action of sodium hydrate on red blood cells, p. 21 ; action of quinine, hydrochloric acid and sodium hydrate on red blood cells compared, p. 22 ; nature of the action of quinine in alkaloidal state on red blood cells, rate of action of alkaloidal quinine, value of  $\mu$ , comparison with pepsin, amount of quinine taken up by red blood cells, p. 27 ; action of quinine on healthy red blood cells in the living body, p. 42.

(b) Action of quinine on red blood cells during blackwater fever, p. 44.

(c) Action of quinine on red blood cells affected with malarial parasites, p. 49.

(d) Action of urine on red blood cells and on dissolved haemoglobin: comparative experiments, p. 51; determination of reaction rate, value of  $\mu$ , p. 55.

(e) Summary, p. 60.

## SECTION II. The relation of haemolysinaemia to the haemoglobinuria of blackwater fever.

Introduction, p. 62; observations made during health, blackwater fever and other affections, p. 66; summary, p. 68.

## SECTION III. The relation of haemoglobinaemia to haemoglobinuria.

Introduction, p. 69.

Is haemoglobinaemia present in blackwater fever? p. 70; is haemoglobinaemia in blackwater fever accompanied by haemoglobinuria? p. 76.

Is haemoglobinuria readily producible as the result of haemoglobinaemia? p. 80; relation between the degree of haemoglobinuria and that of haemoglobinaemia in rabbits, p. 81; relation between the degree of haemoglobinuria and that of haemoglobinaemia in blackwater fever, p. 86; comparison between the amount of dissolved haemoglobin injected in rabbits and the quantity appearing in the urine, p. 89; rate of disappearance in the living body of dissolved haemoglobin from the blood plasma, p. 89; rate of disappearance in vitro of dissolved haemoglobin from the blood plasma, p. 90.

Summary, p. 92.

## SECTION IV. The mechanism of suppression of urine in blackwater fever.

Introduction, p. 94.

Solid matter in the urine: in experimental haemoglobinuria and in redwater, p. 94; in blackwater fever during haemoglobinuria, p. 98; during suppression of urine following blackwater fever, p. 103.

Amount of urine passed during suppression, other characters, Case 16, p. 106.

Condition of kidneys during suppression of urine following blackwater fever, macroscopic and microscopic, diagrammatic representation, p. 107; Case 16, p. 119.

Condition of kidneys of rabbit during haemoglobinuria dependent upon haemoglobinaemia, diagrammatic representation, p. 124; of cattle and dogs during redwater, p. 127.

Condition of kidneys of rabbit during anuria produced by poisoning with potassium chlorate and other poisons, and by the injection of haemolytic serum, p. 128.

Mechanism of suppression of urine, p. 131.

Source of urine during suppression, p. 134.

Summary, p. 135.

#### SECTION V. The mechanism of production of blackwater.

Introduction, p. 136.

Condition of haemoglobin in the urine in blackwater fever: dissolved haemoglobin, p. 137; red blood cells, p. 137; estimation of haemoglobin in urine, p. 138; total amount of haemoglobin lost in the urine in blackwater fever, p. 140.

Mechanism of production of blackwater, relation of blackwater to haemoglobinaemia: significance of red blood cells in the urine, p. 141; alternative hypotheses, primary haemoglobinaemia and primary renal haemorrhage, p. 142; primary haemoglobinaemia considered in relation to the phenomena of blackwater fever, p. 142; primary renal haemorrhage considered in relation to the phenomena of blackwater fever, p. 146; conclusion, p. 150.

Situation in which laking of red blood cells may take place in blackwater fever: in the blood stream, p. 150; in internal organs, p. 153; splenic enlargement, p. 153; splenic puncture, p. 154.

General symptoms, p. 160.

Blackwater and malaria, p. 160.

Blackwater and quinine, p. 163.

Summary, p. 165.

Additional note: mechanism of production of haemoglobinuria in piroplasmiasis, p. 165.

SECTION VI. Condition of the kidneys during blackwater fever. Relation of venereal disease to blackwater fever.

Introduction, p. 166; post mortem observations, p. 167; venereal disease, p. 169; summary, p. 169.

SECTION VII. Icterus and blackwater fever, p. 170.

SECTION VIII. Remarks on prophylaxis and treatment of blackwater fever, p. 171.

SECTION IX. Clinical records.

Review of cases of blackwater fever coming under observation, p. 174; Case 1, p. 176; Case 2, p. 178; Case 3, p. 181; Case 4, p. 186; Case 5, p. 188; Case 6, p. 190; Case 6a, p. 191; Case 7, p. 193; Case 7a, p. 195; Case 8, p. 201; Case 9, p. 205; Case 10, p. 207; Case 11, p. 217; Case 12, p. 222; Case 13, p. 225; Case 14, p. 226; Case 14a, p. 229; Case 15, p. 231; Case 16, p. 235; Case 17, p. 239.

Contents, p. 1.

Tables, p. 5.

Illustrations, p. 8.



## TABLES

	PAGE
TABLE 1. Haemolysis of red cells by quinine bihydrochloride dissolved in 0.9% NaCl solution. Duration of experiment three hours. Temperature 37° C. ... ..	15
TABLE 2. Haemolysis of red blood cells by quinine bihydrochloride, in nearly four times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C. ... ..	16
TABLE 3. Haemolysis of red blood cells by quinine bihydrochloride, in nearly fourteen times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C. ... ..	17
TABLE 4. Haemolysis of red blood cells by quinine bihydrochloride dissolved in the blood plasma. Temperature of experiment 37° C. ... ..	18
TABLE 5. Haemolysis of red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C. ... ..	19
TABLE 6. Haemolysis of red blood cells by hydrochloric acid dissolved in 0.9% NaCl solution. Duration of experiment three hours. Temperature 37° C. ... ..	20
TABLE 7. Haemolysis of red blood cells by sodium hydrate dissolved in 0.9% NaCl solution. Duration of experiment three hours. Temperature 37° C. ... ..	21
TABLE 8. Showing the relative amounts of human red cells completely haemolysed in three hours by quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate. ... ..	22
TABLE 9. Haemolysis of red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C. ... ..	23
TABLE 10. Haemolysis of red blood cells by hydrochloric acid dissolved in 0.9% NaCl solution. Duration of experiment three hours. Temperature 37° C. ... ..	24
TABLE 11. Haemolysis of red blood cells by sodium hydrate dissolved in 0.9% NaCl solution. Duration of experiment three hours. Temperature 37° C. ... ..	24
TABLE 12. Comparison of the action on human red blood cells of quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate in the same molecular concentration. The amounts of red cells given were the largest amounts which could be completely haemolysed in three hours at 37° C. ... ..	25
TABLE 13. The rate of haemolysis of red blood cells by a 0.0358% solution of quinine in 0.9% solution of sodium chloride. The ratio of the weight of wet red cells to the amount of quinine present is 12.6 : 1. Temperature of experiment 37° C. ... ..	29
TABLE 14. Reaction rate constant of the experiments recorded in Table 13 re-calculated. ... ..	29



TABLE 15. Comparison of percentages found in Table 13 and those calculated from the value of  $K = 0.0039$ . ... 30

TABLE 16. The rate of haemolysis of red blood cells by quinine in the alkaloidal state. Conditions of experiment as in Table 13. ... 32

TABLE 17. Rate of haemolysis of red blood cells, at different temperatures by a 0.00113 M. (0.0366 %) solution of quinine in the alkaloidal state. ... 34

TABLE 18. Determination of value of  $\mu$  from experiments recorded in Table 17. ... 35

TABLE 19. Comparison of values of  $K$  at different temperatures... 36

TABLE 20. Determination of the amount of quinine withdrawn from solution by healthy human red blood cells. Duration of action of red blood cells on quinine sulphate dissolved in 0.9 % solution of sodium chloride, eighteen to twenty-four hours. Temperature of experiment 17° C. to 19° C. ... 40

TABLE 21. Determination of the percentage amount of quinine contained in red blood cells in the experiments recorded in Table 20. 41

TABLE 22. Quinine in relation to blackwater fever. ... 42

TABLE 23. Haemoglobinuria in relation to quinine in animals. 43

TABLE 24. The haemolytic action of quinine bihydrochloride upon red blood cells during and after blackwater fever and malaria. Experiments conducted at 37° C. as in Table I (p. 15). Duration of experiment three hours. ... 46

TABLE 25. The action of urine in breaking up haemoglobin. Duration of experiment four hours. Temperature 37° C. ... 53

TABLE 26. The rate of destruction of haemoglobin in red blood cells by urine at different temperatures. ... 56

TABLE 27. Determination of value of  $\mu$  from experiments recorded in Table 24. ... 59

TABLE 28. Comparison of values of  $K$  at different temperatures. Cp. Table 24. ... 60

TABLE 29. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from blackwater fever. ... 65

TABLE 30. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from malarial and other affections. Normal plasma was also employed in eleven observations. ... 66

TABLE 31. Examination of oxalated blood plasma for the presence of dissolved haemoglobin in healthy individuals. ... 71

TABLE 32. Examination of oxalated blood plasma for the presence of dissolved haemoglobin in patients not suffering from blackwater fever. 72

	PAGE
TABLE 33. Examination of oxalated blood plasma for the presence of dissolved haemoglobin in blackwater fever patients. ... ..	73
TABLE 34. Examination of oxalated blood plasma for the presence of dissolved haemoglobin during the haemoglobinuria of blackwater fever. Selected from Table 33. ... ..	75
TABLE 35. Experimental haemoglobinuria in rabbits. ... ..	78
TABLE 36. Examination of oxalated blood plasma for the presence of dissolved haemoglobin in experimentally induced haemoglobinuria in rabbits. Selected from Table 35. ... ..	81
TABLE 37. The rate of disappearance of dissolved haemoglobin from the blood plasma in the living body of the rabbit. Cp. Table 35.	91
TABLE 38. Decomposition of dissolved haemoglobin in oxalated or defibrinated blood plasma. Temperature of experiment 37° C. ...	93
TABLE 39. Characters of deposit from urine of rabbit during haemoglobinuria experimentally induced. Cp. Tables 35 and 37. ...	97
TABLE 40. Characters of deposit from urine during haemoglobinuria of blackwater fever. ... ..	103
TABLE 41. Characters of deposit from urine during suppression in blackwater fever (Cases 7A and 11). Case 16 is also given in this Table.	105
TABLE 42. Average daily amount of urine passed during suppression of urine in blackwater fever. The colour of the urine was light yellow or amber. ... ..	106
TABLE 43. Characters of the urine in blackwater fever in respect of brown coloration, unchanged haemoglobin in solution and red blood cells. ... ..	136
TABLE 44. Bulk of precipitate obtained on boiling 10 c.c. of slightly diluted urine, containing varying percentages of dissolved haemoglobin. ... ..	140
TABLE 45. Amount of haemoglobin passing into the urine in blackwater fever. ... ..	141
TABLE 46. Examination of blood films in cases of blackwater fever.	151
TABLE 47. Condition of the spleen in blackwater fever. ... ..	154
TABLE 48. Condition of parenchyma of spleen in blackwater fever and malaria. ... ..	155
TABLE 49. Character of constitutional disturbance during blackwater fever and of preceding illness when present. ... ..	161
TABLE 50. Showing the amounts of quinine (given in grains) taken before, during and after the attacks of blackwater fever investigated.	247
TABLE 51. Blackwater fever and venereal disease. ... ..	169
TABLE 52. Synopsis of cases of blackwater fever investigated. ...	248

## ILLUSTRATIONS

	PAGE
FIG. 1. Apparatus employed for testing haemolytic property of blood plasma. ... ..	64
FIG. 2. Experiment 3, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	82
FIG. 3. Experiment 4, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	82
FIG. 4. Experiment 5, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	83
FIG. 5. Experiment 6, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	83
FIG. 6. Experiment 7, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	83
FIG. 7. Experiment 8, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	84
FIG. 8. Experiment 9, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	84
FIG. 9. Experiment 10, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	84
FIG. 10. Blackwater fever, Case 3. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	85
FIG. 11. Blackwater fever, Case 7a. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	85
FIG. 12. Blackwater fever, Case 8. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	85
FIG. 13. Blackwater fever, Case 10. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	86
FIG. 14. Blackwater fever, Case 11. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	86
FIG. 15. Blackwater fever, Case 12. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	86
FIG. 16. Blackwater fever, Case 14. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	87



	PAGE
FIG. 17. Blackwater fever, Case 14a. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	87
FIG. 18. Blackwater fever, Case 15. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	87
FIG. 19. Blackwater fever, Case 16. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	88
FIG. 20. Blackwater fever, Case 17. Percentage of dissolved haemoglobin in blood plasma and urine. ... ..	88
FIG. 21. Hyaline casts in urine of rabbit (Exp. 2, Table 35) four hours after intravenous injection of dissolved haemoglobin. ... ..	95
FIG. 22. Granular and epithelial casts in urine of rabbit (Exp. 3, Table 35) twenty hours after intravenous injection of dissolved haemoglobin. ... ..	95
FIG. 23. Granular and epithelial casts in urine of rabbit (Exp. 5, Table 35) eighty-five minutes after intravenous injection of dissolved haemoglobin. ... ..	96
FIG. 24. Granular and epithelial casts in urine of rabbit (Exp. 6, Table 35) twelve and a half hours after intravenous injection of dissolved haemoglobin. ... ..	96
FIG. 25. Deposit from chocolate coloured urine of blackwater fever, Cases 2 and 5. ... ..	98
FIG. 26. Deposit from porter coloured urine of blackwater fever, Case 3. ... ..	99
FIG. 27. Deposit from amber coloured urine of blackwater fever, Cases 4 and 8. ... ..	99
FIG. 28. Deposit from porter coloured urine of blackwater fever, Case 7. ... ..	100
FIG. 29. Deposit from porter coloured urine of blackwater fever, Case 7a. ... ..	100
FIG. 30. Deposit from porter coloured urine of blackwater fever, Case 15. ... ..	101
FIG. 31. Deposit from porter coloured urine of blackwater fever, Case 17. ... ..	102
FIG. 32. Deposit from light yellow coloured urine of blackwater fever, Case 7a. ... ..	104
FIG. 33. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... ..	108
FIG. 34. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... ..	109
FIG. 35. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... ..	110

	PAGE
FIG. 36. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... ..	111
FIG. 37. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... ..	112
FIG. 38. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... ..	113
FIG. 39. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... ..	114
FIG. 40. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... ..	115
FIG. 41. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... ..	117
FIG. 42. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... ..	118
FIG. 43. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... ..	119
FIG. 44. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... ..	120
FIG. 45. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... ..	121
FIG. 46. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... ..	122
FIG. 47. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... ..	123
FIG. 48. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... ..	124
FIG. 49. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... ..	125
FIG. 50. Section of cortex of kidney fourteen hours after experimental haemoglobinuria had ceased. Rabbit 9, Table 35. ... ..	126
FIG. 51. Section of medulla of kidney fourteen hours after experimental haemoglobinuria had ceased. Rabbit 9, Table 35. ... ..	127
FIG. 52. Section of cortex of kidney of bull during redwater due to piroplasmosis. ... ..	128
FIG. 53. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... .. <i>opposite</i>	131
FIG. 54. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater fever, Case 7a. ... .. <i>opposite</i>	131
FIG. 55. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... .. <i>opposite</i>	131

	PAGE
FIG. 56. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater fever, Case 11. ... .. <i>opposite</i>	131
FIG. 57. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... .. <i>opposite</i>	131
FIG. 58. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater fever, Case 16. ... .. <i>opposite</i>	131
FIG. 59. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... .. <i>opposite</i>	131
FIG. 60. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. ... .. <i>opposite</i>	131
FIG. 61. Diagrammatic representation of the condition of the kidney in health, during experimental haemoglobinuria of rabbit and in suppression of urine following upon blackwater fever. ...	129
FIG. 62. Diagrammatic representation of the condition of the glomerular capsule, uriniferous tubule and collecting tubule in health, during experimental haemoglobinuria of rabbit and in suppression of urine following upon blackwater fever. ... ..	130
FIG. 63. Spleen smear. Blackwater fever, Case 7a. ... ..	156
FIG. 64. Spleen smear. Blackwater fever, Case 11. ... ..	156
FIG. 65. Spleen smear. Blackwater fever, Case 12. ... ..	157
FIG. 66. Spleen smear. Blackwater fever, Case 14. ... ..	158
FIG. 67. Spleen smear. Blackwater fever, Case 15. ... ..	158
FIG. 68. Spleen smear. Blackwater fever, Case 16. ... ..	159
FIG. 69. Spleen smear. Malaria. ... ..	159
FIG. 70. Spleen smear. Piroplasmosis of calf.... ..	160
FIG. 71. Blackwater fever, Case 2. Temperature chart. ... ..	180
FIG. 72. Blackwater fever, Case 3. Temperature chart. ... ..	181
FIG. 73. Blackwater fever, Case 7. Temperature chart. ... ..	193
FIG. 74. Blackwater fever, Case 7a. Temperature chart. ... ..	197
FIG. 75. Blackwater fever, Case 10. Temperature chart. ... ..	208
FIG. 76. Blackwater fever, Case 11. Temperature chart. ... ..	218
FIG. 77. Blackwater fever, Case 13. Temperature chart. ... ..	225
FIG. 78. Blackwater fever, Case 14. Temperature chart. ... ..	227
FIG. 79. Blackwater fever, Case 14a. Temperature chart. ... ..	231
FIG. 80. Blackwater fever, Case 15. Temperature chart. ... ..	235
FIG. 81. Blackwater fever, Case 16. Temperature chart. ... ..	238
FIG. 82. Blackwater fever, Case 17. Temperature chart. ... ..	240



## INTRODUCTION

In the past the investigation of blackwater fever has been essentially clinical. The symptoms and course of the disease have been studied and, as regards aetiology, its relation to the administration of quinine has been established; but purely clinical observation has failed to explain the significance of its association with malaria, although its dependence upon malaria has been assumed by most writers. But little attention has been given to the mechanism of production of blackwater fever, although recent advances in experimental physiology and pathology, and above all in physical chemistry, have provided numerous methods applicable to research in this direction. In the investigation which we have made upon blackwater fever, we have endeavoured to trace out some of the internal processes, whose terminal event is the appearance of blackwater, believing that in this way many obscure points in connection with the causation and treatment of this condition would sooner or later be cleared up. In our work we have always attempted to give our observations a quantitative character, and to be as far as possible free from the limitation imposed by purely qualitative methods.

Some of the investigations which we have entered upon have necessarily been of a somewhat tentative character. Thus before the direct action of quinine upon the red blood cells in blackwater fever could be determined, it has been essential first of all to determine its action upon the red blood cells of healthy individuals. The next step undertaken was that of determining if haemolysins, present in the blood, played any part in the production of blackwater, such as is known to be the case in paroxysmal haemoglobinuria. A further point has been the investigation of the relationship of haemoglobin-aemia to haemoglobinuria. Again, in studying the condition of the urine in blackwater fever, it has been found desirable to estimate, whenever possible, the amount of blood cells corresponding to the

dissolved haemoglobin present in the urine, and for this purpose it has also been necessary to investigate quantitatively the action of urine upon haemoglobin. Further light upon the mechanism of production of blackwater fever has also been obtained by studying redwater and by experimentally producing haemoglobinuria in animals.\*

The attacks of blackwater-fever which we have investigated have been twenty in number, and were kindly brought under our observation by the Nyasaland Government medical staff and by the medical officers of the Shiré Highlands Railway Company.

Our thanks are also due to the Nyasaland Government for providing us with rooms at the Hospital at Blantyre.

Our report was completed in the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.

## 1. THE HAEMOLYSIS OF RED BLOOD CELLS BY QUININE AND ALSO BY ACID, ALKALI AND URINE.

### *a. The action of quinine, acid and alkali on healthy red blood cells.*

#### 1. *Action of quinine bihydrochloride on red blood cells in varying concentrations.*

In testing the haemolytic action of quinine† upon red blood cells the bihydrochloride was used because its greater solubility in water permitted the employment of a wider range of concentration than would have been possible had the monochloride or sulphate been chosen. In the first three series of experiments the action of the

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\* See Table of Contents, p. 1.

† The only work on this subject appears to be that of Nocht, *Über Schwarzwasserfieber*, Verhandlungen des deutschen Kolonialkongresses, 1905, who stated that 2 mgrm. of quinine in 5 c.cm. of a 10 per cent. dilution of defibrinated blood in a 0.9 per cent. sodium chloride solution was the maximum amount which could be added short of causing haemolysis.

quinine salt was tested upon red blood cells freed from plasma, the method of procedure in these, and most of the succeeding experiments of the same type, being as follows:—

*Method.*—About one cubic centimetre of blood was obtained by pricking the finger, previously washed and cleansed with absolute alcohol, and allowing blood to flow drop by drop into a small glass collecting tube containing a measured amount of a 1 per cent. solution of potassium oxalate (containing also 0.45 per cent. of sodium chloride, so as to be isotonic with the red cells), in the proportion of one part of the latter to from four to ten parts of blood. The mixture thus obtained was next sucked up into a graduated 2 c.cm. pipette and its volume noted. The percentage (by volume) of red cells contained in the mixture was then determined by means of a haemocrit. The mixture, or a measured fraction of it, was next added to about 10 c.cm. of 0.9 per cent. solution of sodium chloride and centrifugalised until the red cells were completely precipitated. The supernatant fluid was then pipetted off and as much of the 0.9 per cent. solution of sodium chloride added as was required to make up a 2.5 per cent. emulsion of red cells. In many of the experiments a portion of this emulsion was laked by the addition of distilled water, and the amount of haemoglobin it contained determined by means of a haemoglobino-meter reading, the absolute values of the scale of the instrument having previously been determined in terms of the red cells in the moist condition of one of us, taken as a standard. In this way the value of different blood emulsions made up to 2.5 per cent. of wet red cells can be referred, as regards their haemoglobin content, to the same standard. It may be observed that sometimes, as for example in cases of malaria, as will be seen later (cp. Table 24), this is absolutely necessary if different experimental results are to be compared.

The red blood cell emulsion having been prepared as above described measured amounts were then added to a series of test-tubes containing a quinine solution isotonic with blood plasma and, if necessary, also 0.9 per cent. of sodium chloride, so as to make up a diminishing series of concentrations of quinine salt, while at the same time the percentage of wet red cells in the series progressively increased (Table 1). In this way the point was determined at which the red blood cells present were completely haemolysed at the end



of the period of experiment, namely, three hours, the temperature of experiment being 37° C. In Table 1, only the concentration of quinine salt and the ratio of the weight of wet red cells to the weight

TABLE 1. Haemolysis of red blood cells by quinine bihydrochloride dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION				
	Quinine bihydrochloride	0.080 %	0.065 %	0.054 %	0.045 %
	Weight of wet red blood cells	5.2	6.5	8.5	10.4
	Weight of quinine salt	1	1	1	1
1	Complete	Complete	Complete	Complete	Partial
2	Complete	Complete	Complete	Complete	Marked
3	Complete	Complete	Complete	Complete	Partial
4	Complete	Complete	Complete	Complete	Partial
5	Complete	Complete	Complete	Complete	Partial

of quinine present are given, the actual quantities of red blood cell emulsion, quinine solution and of salt solution, which were employed, being omitted so as not to overburden the Table with such details of experiment as are best arranged in accordance with the actual conditions of working which individual experimenters may regard as most convenient. In some of the later experiments, however, the concentration of the solutions employed and the amounts of each used are given (Table 24); from these the reader will readily devise convenient concentrations and quantities to employ in those experiments in which these details are omitted.

During the course of the experiments the tubes containing the mixtures of red blood cell emulsion and quinine solution were stirred with a glass rod every fifteen minutes. In the tubes in which complete haemolysis appeared to have occurred, a microscopical examination of the deposit after centrifugalisation was made in order to make certain that this was the case.

In all these experiments the glass tubes and pipettes used were kept scrupulously clean. From time to time a search for bacteria was made at the close of the experiment, but uniformly with negative result. Actual sterilisation of the glass apparatus and of the solutions employed was carried out only in a few cases in which the time elapsing between the collection of blood and the termination of the experiment exceeded four hours.

On reference to Table 1 it will be seen that haemolysis was complete up to a concentration of 0.045 per cent. of quinine bihydrochloride, at which concentration the weight of red cells haemolysed was 10.4 times that of the quinine salt employed. When a concentration of 0.038 per cent. was employed, the weight of red cells being now 13.7 times that of the quinine salt present in solution, haemolysis was only partial, and on extending the series beyond the limit given in the Table, the red cells were found not to be appreciably affected. The transition point was sharply marked, though slight variations in the rate at which haemolysis proceeds were to be observed. As will appear later (cp. Table 18), in different samples of red blood cells, taken from the same (healthy) individual, similar variations, inconsiderable in extent, occur.

In the more concentrated solutions of quinine bihydrochloride haemolysis proceeds very rapidly. In the first two concentrations given in Table 1, haemolysis was complete within an hour, and in the third at the end of about two hours.

A second series of experiments (Table 2) was next carried out, in which the concentration of the quinine salt at the transition point was nearly four times that present at the transition point of the first series. In these, as in the former experiments, the transition point

TABLE 2. Haemolysis of red blood cells by quinine bihydrochloride, in nearly four times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION				
	Quinine bihydrochloride 0.223 %	0.188 %	0.155 %	0.134 %	0.119 %
	Weight of wet red blood cells 10.4	13.0	15.6	18.2	20.8
	Weight of quinine salt 1	1	1	1	1
1	Complete (Marked)*	Complete (Partial)	Marked (Slight)	Marked (Slight)	Slight (Slight)
2	Complete (Complete)	Complete (Marked)	Marked (Slight)	Slight (Slight)	Slight (Slight)
3	Complete (Complete)	Complete (Complete)	Complete (Slight)	Marked (Slight)	Slight (Slight)
4	Complete (Complete)	Complete (Complete)	Complete (Complete)	Marked (Slight)	Slight (Slight)
5	Complete (Complete)	Complete (Complete)	Complete (Marked)	Slight (Slight)	Slight (Slight)

\* The results in brackets represent the action of quinine bihydrochloride when, instead of washed red cells, equivalent amounts of oxalated blood are used.



is well marked, and occurs when a concentration of quinine bihydrochloride amounting to 0.155 per cent. is reached. It will be noticed that in two members of the series, haemolysis, at the transition point, was nearly but not quite complete at the end of three hours at 37° C., the rate at which the reaction proceeded being in these two cases slightly slower than in the remaining members of the same group. In the succeeding concentration [0.134 per cent.] of quinine salt haemolysis is partial or slight, and in the remaining concentration (0.119 per cent.) but little haemolysis occurs. In these experiments it is seen that a given weight of quinine bihydrochloride does not haemolyse a fixed weight of red blood cells during the period of experiment (three hours), but that the maximum weight of red blood cells, which can be haemolysed, depends upon the concentration of the quinine salt, not upon its absolute amount.

A third series of experiments was now carried out with still higher concentrations of quinine salt (Table 3). Here, as before, the transition point was well marked. It was obtained when a concentration of quinine salt amounting to 0.635 per cent., i.e. about fourteen times that obtaining in the first series [0.045 per cent.] and nearly

TABLE 3. Haemolysis of red blood cells by quinine bihydrochloride, in nearly fourteen times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.					
	Quinine hydrochloride	0.844 %	0.724 %	0.635 %	0.527 %	0.448 %
	Weight of wet red blood cells	22	29	36.4	46	57
	Weight of quinine salt	1	1	1	1	1
1	Complete	Complete	Complete	Partial	Slight	
2	Complete	Complete	Marked	Partial	Slight	
3	Complete	Complete	Complete	Marked	Slight	
4	Complete	Complete	Complete	Partial	Slight	
5	Complete	Complete	Complete	Partial	Slight	

two and a half times that present in the second series [0.155 per cent.], was reached. The weight of the red blood cells haemolysed was 36.5 times that of the quinine salt present. As in the second series, so again here, the increase in the amount of red blood cells haemolysed is not in the same proportion as is that of quinine salt present, but is much smaller than the latter.

Up to the present only washed red blood cells, freed from plasma, have been considered. In Table 2, however, side by side with the results obtained with washed red cells, are given, in brackets, those obtained with the same amounts of red cells not freed from plasma. The haemolysis in the latter case is not so marked as in the former, the transition point being reached when a concentration of quinine bihydrochloride amounting to 0.188 per cent. is attained (as against 0.155 per cent. in the former series), and the weight of red blood cells haemolysed is thirteen times that of the quinine salt present (as against 15.6 in the former series), some of the quinine being apparently taken up by the plasma and thus prevented from acting upon the red blood cells. There is also noticeable, less regularity in this series, when compared with the former. This is perhaps attributable to diurnal variations in the composition of the plasma.

In Table 4 the action of quinine bihydrochloride upon red blood cells contained in more or less diluted oxalated plasma is shown. In

TABLE 4. Haemolysis of red blood cells by quinine bihydrochloride dissolved in the blood plasma. Temperature of experiment 37° C.

No. of Experiment	Source of blood	COMPOSITION OF MIXTURE OF OXALATED BLOOD AND SOLUTION OF QUININE BIHYDROCHLORIDE			Condition of mixture at end of experiment
		blood	Q.2HCl	$\frac{\text{weight of wet red cells}}{\text{weight of Q.2HCl}}$	
1	Human	96 %	0.21 %	195 : 1	Plasma contained 0.6 % of dissolved haemoglobin at the end of three hours.
2	"	92 %	0.41 %	96 : 1	" 0.4 % "
3	"	90 %	0.50 %	77 : 1	" 0.8 % "
4	"	89 %	0.54 %	71 : 1	" 1.8 % "
5	"	88 %	0.57 %	67 : 1	" 2.5 % "
6	"	88 %	0.58 %	64 : 1	" 3.1 % "
7	"	87 %	0.62 %	60 : 1	" 5.7 % "
8	"	87 %	0.62 %	60 : 1	" 6.8 % "
9	"	84 %	0.81 %	45 : 1	" 22.5 % "
10	"	50 %	0.53 %	42 : 1	Red cells completely haemolysed at end of 7 hours.
11	Rabbit	42 %	0.96 %	17 : 1	Red cells completely haemolysed at end of 15 mins.

these experiments the blood employed formed from 42 per cent. to 96 per cent. of the mixture. As in the preceding experiments the protective action of the plasma is well marked. Thus in Experiment 9, in which 84 per cent. of blood is present and the concentration of

quinine bihydrochloride is 0.81 per cent., complete haemolysis was not attained after the lapse of three hours, while in Table 3, with a concentration of the quinine salt of 0.635 per cent. haemolysis was complete in three hours, the ratio of the weight of red blood cells to that of quinine bihydrochloride being, in the first case, about 45 to 1, and, in the second case, 36.4 to 1.

2. *Action of quinine in the form of alkaloid on red blood cells.*

So far the action upon red blood cells of quinine in the form of a salt has been investigated. Since quinine has not, however, been shown to be present in the blood in the form of a quinine salt, but may possibly be present in the free state, it was therefore decided to investigate the action of quinine in the alkaloidal form (Table 5). These experiments, owing to the sparing solubility of alkaloidal quinine, namely one in about sixteen hundred parts of water at 15° C., could only be carried out in very dilute solution. The series of experiments given in Table 5 is similar to those given in Table 1, except that the free alkaloid is employed instead of the quinine salt. Here it is seen that the amount of haemolysis is less than when the quinine salt was employed, the transition point, which as before is sharply marked, occurring with a concentration of alkaloid of 0.063 per cent., which is equivalent to 0.077 per cent. of quinine bihydrochloride, while the weight of red blood cells completely haemolysed is six and a half times that of the free quinine employed, or five and a quarter times that of the corresponding amount of quinine bihydrochloride.

TABLE 5. Haemolysis of red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.				
	Quinine (alkaloid)	0.078 %	0.063 %	0.052 %	0.045 %
	Weight of wet red blood cells	4.8	6.4	8.0	9.6
	Weight of quinine (alkaloid)	1	1	1	1
1	Complete	Complete	Partial	Partial	Trace
2	Complete	Complete	Partial	Slight	Trace
3	Complete	Complete	Partial	Partial	Partial
4	Complete	Complete	Marked	Marked	Trace
5	Complete	Marked	Marked	Partial	Partial



It may here be pointed out that in these experiments quinine is present in colloidal solution, and that film formation on the liquid-air surface of the quinine solution is very marked. If such a solution is several times poured from one vessel to another a large amount of the quinine present may be separated out, and the concentration of the alkaloid correspondingly lowered. It is, therefore, necessary to avoid as far as possible any procedure likely to cause such separation of quinine. After the admixture of the solution of alkaloidal quinine with emulsion of red blood cells, the tendency to film formation is no longer observable.

### 3. *Action of hydrochloric acid on red blood cells.*

The quinine salt employed in the experiments recorded in Tables 1 to 3, when dissolved in water, becomes in part hydrolysed and forms an acid solution. It becomes therefore desirable to institute a comparison between the action of hydrochloric acid and of quinine upon red blood cells. In this connection, in order to admit of a wider range of comparison, the action of free alkali was also determined and may thus be contrasted with that of quinine in the form of free base. The latter experiments are given in the succeeding section.

TABLE 6. Haemolysis of red blood cells by hydrochloric acid dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND HYDROCHLORIC ACID SOLUTION.				
	Hydrochloric acid 0.0137 %	0.0114 %	0.0098 %	0.0086 %	0.0076 %
	Weight of wet red cells 146	182	219	255	292
	Weight of hydrochloric acid 1	1	1	1	1
1	Complete	Complete	Partial	Slight	Slight
2	Complete	Complete	Partial	Slight	Slight
3	Complete	Complete	Marked	Slight	Slight
4	Complete	Complete	Complete	Slight	Slight
5	Complete	Complete	Marked	Slight	Slight

The experiments made with hydrochloric acid, which was dissolved in 0.9 per cent. sodium chloride solution, so as to be isotonic with blood plasma, are given in Table 6. These experiments form a continuation of those given in Table 2, with hydrochloric acid in place of quinine bihydrochloride. The action of hydrochloric acid in causing

haemolysis is effective in much weaker concentration than is quinine bihydrochloride, the transition point being reached with 0.0114 per cent. of hydrochloric acid, while the corresponding weight of red blood cells haemolysed was one hundred and eighty-two times that of the hydrochloric acid present. The action of hydrochloric acid was not confined to the liberation of haemoglobin from the red blood cells. The liberated haemoglobin quickly underwent a further change, a soluble substance of brown colour, much more stable than haemoglobin, but giving no absorption bands on spectroscopic examination, making its appearance.

#### 4. *Action of sodium hydrate on red blood cells.*

The action of caustic alkali on red blood cells differs from that of the preceding haemolytic agents, inasmuch as with it the transition point at which complete haemolysis ceases is not sharply defined, as will be seen on reference to Table 7, which, like Table 6, is a continuation of the series of experiments given in Table 2, but with sodium

TABLE 7. Haemolysis of red blood cells by sodium hydrate dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND SODIUM HYDRATE SOLUTION.				
	Sodium hydrate	0.021 %	0.018 %	0.016 %	0.015 %
	Weight of wet red cells	113	133	153	166
	Weight of sodium hydrate	1	1	1	1
1	Complete	Almost complete	Marked	Marked	Marked
2	Complete	Almost complete	Marked	Marked	Marked
3	Complete	Complete	Complete	Complete	Marked
4	Complete	Complete	Complete	Complete	Marked
5	Complete	Complete	Complete	Complete	Marked

hydrate substituted for quinine bihydrochloride. In these experiments haemolysis was complete as far as a concentration of about 0.015 per cent. (chemically equivalent to 0.145 per cent. of quinine bihydrochloride). At this concentration the weight of red blood cells haemolysed was one hundred and sixty-six times the weight of sodium hydrate present. Thus, while the concentration of sodium hydrate in Table 7 is only one-tenth of that of quinine bihydrochloride in



Table 2, yet the weight of red cells haemolysed by sodium hydrate is nearly ten times that haemolysed by the quinine salt during the same period of time at the same temperature.

5. *Action of quinine, hydrochloric acid and sodium hydrate on red blood cells compared.*

In the preceding observations the concentration of the various haemolytic agents has been given in percentage terms. For the sake of comparison all these results are grouped together in Table 8. The action of these agents, if it be of a chemical nature, will be a molecular action, and in this case the concentrations employed can be compared only if they are expressed in molar form. In Table 8, therefore, the molar concentration is given by the side of the percentage concentration, and similarly the maximum weight of red blood cells haemolysed is given not only in terms of the absolute weight of the haemolytic agent present, taken as a unit, but also in terms of a gramme-molecule of the latter, taken as a unit. In this Table, in addition to the results already given in the first seven Tables, an additional concentration of sodium hydrate at the transition point is also given, but it has not been thought necessary to give the complete table from which this result has been taken, for a further series of experiments, with slightly lower concentrations, is given in Table 11.

TABLE 8. Showing the relative amounts of human red cells completely haemolysed in three hours by quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate.

Table	Haemolytic agent	Concentration of haemolytic agent	Weight of wet red cells : Weight of haemolytic agent	Wet red cells (in grammes) : Haemolytic agent (gramme-molecule)
1	Q. 2 HCl	0.0450 % = 0.00113 M	10.4 : 1	4130 : 1
2	"	0.1550 % = 0.00389 M	15.6 : 1	6200 : 1
3	"	0.6350 % = 0.01600 M	36.4 : 1	14430 : 1
5	Q. (alkaloid)	0.0628 % = 0.00198 M	6.4 : 1	2070 : 1
6	HCl	0.0114 % = 0.00320 M	182.0 : 1	6640 : 1
7	NaOH	0.0157 % = 0.00380 M	166.0 : 1	6640 : 1
	"	0.0090 % = 0.00226 M	133.0 : 1	5320 : 1

If the solutions employed to produce haemolysis are compared in respect of actual percentage of haemolytic agent present, it will be seen that hydrochloric acid is by far the most potent of the four made use of. Sodium hydrate comes close to hydrochloric acid, while quinine, even in higher concentrations, is a relatively feeble haemolytic agent, especially if employed in the alkaloidal state. When, however, the maximum weight of red blood cells completely haemolysed is compared, not with the actual weight of haemolytic agent present, but instead, with the weight of red blood cells haemolysed by a gramme-molecule of the haemolytic agent, then it is seen that, molecule for molecule, the above difference largely disappears, and the weight of wet red blood cells haemolysed varies in the different experiments within somewhat narrow limits. The results collected together in Table 8 are not, however, altogether suitable for close comparison since the molecular concentrations in the different series are not identical. In order to permit of a closer comparison, three additional series of experiments were carried out with

TABLE 9. Haemolysis of wet red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.				
	Quinine (alkaloid)	0.0366 %	0.0366 %	0.0366 %	0.0366 %
	Weight of wet red cells	2.4	3.2	4.8	6.4
	Weight of quinine (alkaloid)	1	1	1	1
1	Complete	Complete	Marked	Partial	Slight
2	Complete	Complete	Complete	Marked	Marked
3	Complete	Complete	Complete	Very Marked	Partial
4	Complete	Complete	Complete	Marked	Slight
5	Complete	Complete	Complete	Almost complete	Marked

quinine in the free state, hydrochloric acid and sodium hydrate, each series having the molar concentration 0.00113, and thus possessing the concentration of the transition point in Table 1. These experiments are given in Tables 9, 10 and 11, and the concentrations at the transition points are collected together in Table 12. No further reference is required at this point to Tables 9, 10 and 11. In Table 12 it is seen that, when equimolecular concentrations are employed, the weight of red blood cells completely haemolysed in three hours

at 37° C. is in every case nearly the same, varying within very narrow limits, hydrochloric acid and sodium hydrate haemolysing in the concentration, given respectively 3,220 and 4,520 parts by weight of

TABLE 10. Haemolysis of red blood cells by hydrochloric acid dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND HYDROCHLORIC ACID				
	Hydrochloric acid	0.0041 %	0.0041 %	0.0041 %	0.0041 %
	Weight of wet red cells	54.7	72.9	91.1	109
	Weight of hydrochloric acid	1	1	1	1
1	Complete	Complete	Complete	Slight	Trace
2	Complete	Complete	Complete	Slight	Trace
3	Complete	Complete	Complete	Slight	Trace

wet red blood cells per gramme-molecule of haemolytic agent, quinine in the alkaloidal form 1,550, and quinine bihydrochloride 4,130 parts of red cells. Quinine in the alkaloidal form, as already mentioned, is in colloidal solution in water. The low figure, which it gives, as compared with hydrochloric acid and sodium hydrate, which are in true solution and nearly completely ionised, may possibly be in part determined by the physical condition of hydrosol in which the

TABLE 11. Haemolysis of red blood cells by sodium hydrate dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND SODIUM HYDRATE				
	Sodium hydrate	0.0045 %	0.0045 %	0.0045 %	0.0045 %
	Weight of wet red cells	93	113	153	173
	Weight of sodium hydrate	1	1	1	1
1	Complete	Complete	Marked	Slight	Trace
2	Complete	Complete	Almost complete	Marked	Trace
3	Complete	Almost complete	Marked	Slight	Trace

alkaloidal quinine exists. On the other hand quinine bihydrochloride is in part hydrolysed, so that we have here to do with a triple effect, the solution being in reality a mixture of quinine salt, quinine in the



alkaloidal form, and hydrochloric acid. On this account the production of a relatively greater degree of haemolysis is to be expected.\* Taking all these facts into consideration, the results in Table 12 certainly suggest that in all cases the same process is at work, in other words, that whether haemolysis is caused by quinine salt, by quinine in the alkaloidal state, by hydrochloric acid, or by sodium hydrate, an identical reaction occurs. This point will be further referred to in the next section.

In addition to their haemolytic power the four substances in question may also be compared in respect of several other features. In the first place the sharpness of the transition point may be considered. This, as has already been mentioned, is well defined for quinine bihydrochloride, quinine in the free state and hydrochloric acid. If the series of tubes, in which the mixtures of haemolytic agent and red blood cells are contained, is examined at the end of three hours at 37° C., it is found that haemolysis is complete in all tubes up to the transition point. In the next tube there is more or less incomplete haemolysis. In the succeeding tube the haemolysis is slight, and in further tubes the supernatant liquid, left after centrifugalising, shows no indication of laking recognisable with the naked eye. With sodium hydrate, however, although the transition point can be recognised, there is no sudden cessation of haemolysis in the tubes lying beyond this point, but instead the degree of haemolysis lessens very gradually, most of the red cells present in the fifth or sixth tubes beyond that of the transition point being generally haemolysed.

TABLE 12. Comparison of the action on human red blood cells of quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate in the same molecular concentration. The amounts of red cells given were the largest amounts which could be completely haemolysed in three hours at 37° C.

Table	Haemolytic agent	Concentration of haemolytic agent	Weight of wet red cells : Weight of haemolytic agent	Wet red cells (in grammes) : haemolytic agent (gramme-molecule)
I	Q. 2 HCl	0.0450 % = 0.00113 M	10.4 : 1	4130 : 1
9	Q. (alkaloid)	0.0366 % = 0.00113 M	4.8 : 1	1550 : 1
10	HCl	0.0041 % = 0.00113 M	91.1 : 1	3220 : 1
11	NaOH	0.0045 % = 0.00113 M	113 : 1	4520 : 1

\* In 0.00113 M concentration quinine bihydrochloride is hydrolysed to the extent of about ten per cent.

A further difference between the haemolytic agents lies in their action in producing a further change in the haemoglobin liberated from the red cells. Hydrochloric acid produces the most marked effect, rapidly and, in the stronger solutions, in Table 6, completely changing the liberated haemoglobin, so that a brown solution results, which, as already mentioned, gives, on spectroscopic examination, no absorption bands. In less concentrated solutions the appearance of the brown coloration is less marked, but continues to increase when the tube is kept. Quinine bihydrochloride also produces the same change, especially in the more concentrated solutions which were employed, but in much less degree. In the more concentrated solutions of sodium hydrate also the brownish coloration is produced. On the other hand, quinine in the free state produces little change of this kind, the tubes in which it is contained not turning appreciably brown. Moreover, emulsions of red blood cells laked by free quinine exhibit little difference, as far as the haemoglobinometer reading is concerned, from emulsions of the same strength laked with distilled water, and the haemoglobin set free from red blood cells by alkaloidal quinine changes very slowly on keeping, so that the haemoglobinometer reading does not vary during the period of three hours to a degree sufficient to vitiate the experiment, as is the case with the remaining three haemolytic agents. Quinine bihydrochloride was found to cause the appearance of a relatively small amount of methaemoglobin.

A third point of considerable interest bearing on the nature of the process of haemolysis lies in the amount of haemolysis which the different concentrations of the haemolytic agents are capable of producing. Table 8 shows that the weight of red blood cells completely haemolysed in three hours by quinine bihydrochloride is approximately proportional to the square root of the concentration, for on reference to Experiments 1, 2 and 3 it is seen that the weight of red cells is 10.4, 15.6 and 36.4 that of the quinine salt, while the respective concentrations of the latter are 0.045 per cent., 0.155 per cent. and 0.635 per cent. If the weight of red blood cells completely haemolysed were exactly proportional to the square roots of the concentration of quinine salt, the first figures would be 10.4, 18.5 and 39.3 respectively, a difference which is well within the range of experimental error. For the concentrations of hydrochloric acid and



sodium hydrate employed, which are well in the neighbourhood of the lowest concentration of quinine bihydrochloride, the weight of red blood cells completely haemolysed per unit weight of the haemolytic agent is also approximately proportional to the square root of the latter. Thus in the case of hydrochloric acid the maximum weight of wet red blood cells completely haemolysed in three hours in concentrations of 0.00114 per cent. and 0.0041 per cent. respectively is 182 and 91, the calculated amounts being 153 and 91. Similarly with concentrations of sodium hydrate of 0.0157 per cent., 0.0090 per cent. and 0.0045 per cent. the maximum weights of red blood cells completely haemolysed in three hours at 37° C. were respectively 166, 133 and 113, per unit weight of the haemolytic agent, the calculated amounts being 163, 136 and 113. In the case of quinine in the free state, although here, in consequence of the sparing solubility of the alkaloid and the limited range of concentration available, it has not been possible to determine this point with sufficient accuracy to enable an altogether satisfactory proof of the relationship between concentration and haemolytic power to be given, the concentrations employed being somewhat close together and the experimental error relatively large in consequence, yet the figures obtained—6.4 and 4.8 for the red cells and 0.0628 per cent. and 0.0366 per cent. for the concentration—indicate that the former is proportional to the square root of the latter, the calculated amounts for the red cells being 5.8 and 4.8 respectively.

#### 6. *Nature of the action of quinine in the alkaloidal state on red blood cells.*

When haemoglobin is set free the action of the haemolytic agent is not directed to the red cell as a whole, for the cell is not chemically homogeneous, but to one of its constituents. Whether this constituent is haemoglobin itself or is part of the stroma, as in the case of haemolysins produced as antibodies by the injection of red cells into a foreign organism,\* is not determined by our further experiments, which have instead been directed to the problem, what is the nature of the action of the haemolytic agents which have been used in the

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\* Ivar Bang und J. Forssman, Untersuchungen über die Hämolysinbildung. Zeitschr., f. d. ges. Biochemie, 1906. Bd. VIII, S. 238.

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preceding investigation, in particular, is their action a chemical action, or does a purely physical process take place?

With a view to finding an answer to this enquiry, it was decided to determine the reaction rate at which haemolysis proceeds in the heterogeneous system formed by red blood cells suspended in liquid containing a haemolytic agent. This was found to be possible only in the case of quinine in the alkaloidal state, the remaining three haemolytic agents causing, in addition to haemolysis, decomposition of the liberated haemoglobin, and thus preventing the estimation of the amount liberated from being carried out.

The mode of procedure finally adopted for the determination of the reaction rate of quinine in the alkaloidal state was as follows:—

*Method.* About 100 c.cm. of an emulsion of washed red cells in 0.9 per cent. sodium chloride solution containing 0.0358 per cent. = 0.00108 M. of quinine was prepared. This was placed in a water bath kept within  $\pm 0.5^\circ$  C. of the temperature selected for experiment. The emulsion was shaken up every fifteen minutes and at the end of an hour, and subsequently at half hourly intervals, samples of the emulsion measuring about 10 c.cm. were pipetted off and the percentage of haemoglobin (in terms of the equivalent amount of healthy red cells in the moist condition) present in the supernatant liquid after centrifugalisation, diluted if necessary with one to three parts of distilled water, determined by means of a haemoglobinometer, the absolute value of the readings of which had been previously ascertained in terms of the equivalent weight of red cells of one of us taken as a standard. Inasmuch as the laked haemoglobin undergoes a slight deterioration during the course of the experiment, a small correction, representing the mean of several determinations, and amounting to 0.5 division of the haemoglobinometer scale per hour, was applied to the readings. This correction is slight for the earlier haemoglobin estimations, and negligible for the later ones. The haemoglobinometer readings were readily made, the variations between successive readings of the same solution lying within  $\pm 2$  per cent. of the whole, and for readings lying between 25 and 65 on the scale being usually identical.

A series of estimations of the actual and percentage amounts of red blood cells haemolysed in successive periods of time is given in Table 13. The initial concentration of red cells in the emulsion was



0.476 per cent., and the percentage of quinine in the free state 0.0358 per cent. = 0.00108 M. To the results given in the second and third columns the formulae for bimolecular and multimolecular reaction

TABLE 13. The rate of haemolysis of red blood cells by a 0.0358 % solution of quinine in 0.9 % solution of sodium chloride. The ratio of the weight of wet red cells to the amount of quinine present is 12.6 : 1. Temperature of experiment 37° C.

Time $t_x$	Concentration of wet red cells	Same in percentage of 0.476	$\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$
$t_0$ 0 min.	0.476 %	100.0	
$t_1$ 50 "	0.432 %	90.8	0.0009
$t_2$ 80 "	0.396 %	82.0	0.0011
$t_3$ 110 "	0.341 %	71.7	0.0013
$t_4$ 140 "	0.258 %	54.2	0.0019
$t_5$ 170 "	0.188 %	39.5	0.0024
$t_6$ 200 "	0.146 %	30.7	0.0021
$t_7$ 230 "	0.074 %	21.2	0.0029
$t_8$ 260 "	0.029 %	6.1	0.0047
			Mean 0.0022

rates were applied, but the values of K obtained were so divergent that these two reaction rates may be excluded from consideration. In Table 13 the reaction rate constant for monomolecular reactions,  $K = \frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$ , is calculated in the last column. The values obtained range between 0.0009 and 0.0047, the mean being 0.0022. The variations in the value of K are greatest at the beginning and end of the process of haemolysis, being low at the former and high at the latter. If, however, the period  $t_2$  to  $t_7$  is alone considered, the variations are much less, and during this period the reaction follows fairly closely a monomolecular rate.

TABLE 14. Reaction rate constant of the experiments recorded in Table 13 recalculated.

Time	Concentration of wet red cells	Same in percentage of 0.476	$\frac{1}{t_{n+1} - t_n} \log \frac{C_n}{C_{n+1}}$
$t_0$ 0 min.	0.476 %	100.0	
$t_1$ 50 "	0.432 %	90.8	0.0009
$t_2$ 80 "	0.396 %	82.0	0.0015
$t_3$ 110 "	0.341 %	71.7	0.0019
$t_4$ 140 "	0.258 %	54.2	0.0040
$t_5$ 170 "	0.188 %	39.5	0.0045
$t_6$ 200 "	0.146 %	30.7	0.0037
$t_7$ 230 "	0.074 %	21.2	0.0054
$t_8$ 260 "	0.029 %	6.1	0.0183
			Mean $t_2$ to $t_7$ 0.0039

The mode of calculating  $K$  adopted in Table 13, namely by determining  $K$  for a series of periods, each of which commences at the same point of time, that is to say, the time at which the experiment is begun, is well adapted for showing progressive variations in the values of  $K$ , since in this way the irregularities due to error of estimation become less accentuated. The latter are greatest when  $K$  is calculated for every pair of successive estimations, as is done in Table 14, where the values obtained present greater irregularities than in Table 13. The method of calculation adopted in Table 14 gives a more correct average for the period  $t_2$  to  $t_7$ , than is obtainable by the method employed in Table 13. In Table 15 the percentages of red cells haemolysed are calculated from the average value of  $K$  between

TABLE 15. Comparison of percentages found in Table 13 and those calculated from the value of  $K = 0.0039$ .

Time	$t_{n+1} - t_n$	$K (t_{n+1} - t_n)$	Concentration of wet red cells	
			Calculated	Found
$t_0$ 0 min.			[187.2]	[100]
$t_1$ 50 "	50 min.	$0.0039 \times 50 = 0.195 = \log 1.57 = \log \frac{187.2}{119.2}$	[119.2]	[90.8]
$t_2$ 80 "	30 "	" $30 = 0.117 = \log 1.31 = \log \frac{119.2}{91.2}$	[91.2]	[82.0]
$t_3$ 110 "	30 "	" " " $= \log \frac{91.2}{69.5}$	69.5	71.7
$t_4$ 140 "	30 "	" " " $= \log \frac{69.5}{53.1}$	53.1	54.2
$t_5$ 170 "	30 "	" " " $= \log \frac{53.1}{40.5}$	40.5	39.5
$t_6$ 200 "	30 "	" " " $= \log \frac{40.5}{30.9}$	30.9	30.7
$t_7$ 230 "	30 "	" " " $= \log \frac{30.9}{23.6}$	23.6	21.1
$t_8$ 260 "	30 "	" " " $= \log \frac{23.6}{18.1}$	[18.1]	[6.1]

$t_2$  and  $t_7$  obtained in Table 14. It is seen that within this period there is a close correspondence between the calculated and observed percentages. A comparison of the calculated and observed results,

preceding and following this period, which are given in brackets, shows, as is also seen in Tables 13 and 14, the extent to which at the beginning haemolysis proceeds slowly and towards the end progresses with increased rapidity. The cause of this initial delay and final acceleration of the process of haemolysis is not clear; it may perhaps be dependent upon the physical conditions under which the interaction of cell and haemolytic agent occurs. It is, however, established by the above experiments that the reaction in question during the greater part of its course resembles a chemical reaction monomolecular in character, presenting, however, an initial retardation and final acceleration.

In Table 16 the series of experiments given in Table 13 is repeated, and seven other series added. In these the reaction rate constant is calculated for the period  $t_1$  to  $t_8$ , the starting point being  $t_1$  instead of  $t_0$  as in Table 13. This method of calculation has been adopted because of the difficulty of ascertaining the precise moment at which the temperature of the emulsion becomes identical with that of the water in the bath. In these different series of experiments haemolysis is seen to follow pretty closely a monomolecular rate except at the beginning and end of the reaction, when the respective slowing and acceleration already referred to occur, while on the other hand a bimolecular or multimolecular rate is excluded by the much wider range of variation in the value of the constants calculated for such reaction rates. The actual reaction rate as shown by the values of  $K$  varies from day to day even in the same healthy individual, the variations being greater in the series  $Y_1$  to  $Y_4$  than in the series  $B_1$  to  $B_4$ . The cause of these variations, which appear to lie outside the range of experimental error, is obscure. It is true that variations occur in the red blood cells employed in different experiments. In particular, it was constantly found that although the percentage weight of wet red cells in the emulsions was always the same, namely, 0.444 per cent., being determined by means of the haemocrit, yet when the red blood cell content of the emulsions was determined (after laking with distilled water) by means of a haemoglobinometer reading the result was different in each case. Thus in the experiments recorded in Table 16 the haemoglobinometer determinations of the emulsions were found to vary from 0.476 per cent. to 0.532 per cent. These variations are too large to be accounted for as due to

TABLE 16. The rate of haemolysis of red blood cells by quinine in the alkaloidal state. Conditions of experiment as in Table 13.

Time	Y 1				Y 2				Y 3				Y 4			
	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	
$t_0$ 0 min.	0.496%	100.0	—	0.492%	100.0	—	0.524%	100.0	—	0.532%	100.0	—	0.532%	100.0	—	
$t_1$ 50 "	0.435%	87.7	—	0.391%	79.5	—	0.442%	84.2	—	0.417%	85.9	—	0.417%	85.9	—	
$t_2$ 80 "	0.368%	74.2	0.0024	0.275%	55.9	0.0051	0.353%	67.3	0.0017	0.370%	78.0	0.0014	0.370%	78.0	0.0014	
$t_3$ 110 "	0.286%	57.7	0.0030	0.146%	29.7	0.0071	0.252%	48.1	0.0040	0.299%	69.5	0.0015	0.299%	69.5	0.0015	
$t_4$ 140 "	0.215%	43.3	0.0034	0.087%	19.5	0.0068	0.149%	28.4	0.0052	0.266%	56.2	0.0020	0.266%	56.2	0.0020	
$t_5$ 170 "	0.142%	38.7	0.0028	0.048%	9.7	0.0084	0.098%	18.7	0.0054	0.136%	42.4	0.0025	0.136%	42.4	0.0025	
$t_6$ 200 "	0.109%	22.0	0.0032	0.021%	4.3	0.0084	0.062%	11.8	0.0057	0.073%	25.5	0.0034	0.073%	25.5	0.0034	
$t_7$ 230 "	0.058%	11.7	0.0049	0.018%	3.6	0.0084	0.038%	7.2	0.0060	0.040%	13.3	0.0045	0.040%	13.3	0.0045	
$t_8$ 260 "	0.018%	3.6	0.0065	0.015%	3.0	0.0068	0.014%	2.7	0.0071	—	7.5	—	—	—	—	
$t_9$ 290 "	—	—	mean 0.0037	0.003%	0.6	0.0088	0.008%	1.5	mean 0.0073	—	—	mean 0.0026	—	—	—	
		B 1			B 2			B 3			B 4					
$t_0$ 0 min.	0.476%	100.0	—	0.504%	100.0	—	0.496%	100.0	—	0.496%	100.0	—	0.500%	100.0	—	
$t_1$ 50 "	0.432%	90.8	—	0.455%	90.2	—	0.456%	91.8	—	0.456%	91.8	—	0.434%	86.8	—	
$t_2$ 80 "	0.396%	82.0	0.0015	0.410%	81.2	0.0015	0.425%	85.7	0.0010	0.407%	81.4	0.0010	0.407%	81.4	0.0010	
$t_3$ 110 "	0.341%	71.7	0.0019	0.319%	63.2	0.0026	0.372%	75.0	0.0014	0.381%	76.2	0.0009	0.381%	76.2	0.0009	
$t_4$ 140 "	0.258%	54.2	0.0025	0.252%	50.0	0.0028	0.281%	56.7	0.0023	0.315%	63.0	0.0016	0.315%	63.0	0.0016	
$t_5$ 170 "	0.188%	39.5	0.0030	0.177%	35.1	0.0034	0.196%	39.5	0.0030	0.260%	52.0	0.0018	0.260%	52.0	0.0018	
$t_6$ 200 "	0.146%	30.7	0.0031	0.084%	16.7	0.0050	0.127%	25.6	0.0037	0.203%	40.6	0.0023	0.203%	40.6	0.0023	
$t_7$ 230 "	0.074%	21.2	0.0035	0.060%	11.9	0.0049	0.049%	9.9	0.0042	0.134%	26.8	0.0029	0.134%	26.8	0.0029	
$t_8$ 260 "	0.029%	6.1	0.0056	0.033%	6.5	0.0054	0.013%	2.6	0.0073	0.056%	11.0	0.0043	0.056%	11.0	0.0043	
			mean 0.0030			mean 0.0036			mean 0.0033			mean 0.0021				



error of experiment. Moreover, such diurnal variations in health have their counterpart in the much larger variations in disease, as will be seen later in Table 24. Here we have evidence of variation in the content of haemoglobin per unit volume of wet red blood cells which is capable of independent variation in health. When, however, the variations in haemoglobin in different samples of blood from the same individual in Table 16 are compared with corresponding values of  $K$ , it is seen that no constant relationship between the two is recognisable. The mean value of  $K$  at  $37^\circ \text{C}$ . for the Y series is 0.0035, and for the B series 0.0030.

In monomolecular reaction rates at different temperatures the relation

$$\frac{K_2}{K_1} = e^{\frac{\mu}{2} \left( \frac{1}{\tau_1} - \frac{1}{\tau_2} \right)}$$

holds,  $\tau_1$  and  $\tau_2$  being the absolute temperatures at which two estimations of  $K$  are carried out. To ascertain if this relation held for the haemolysis of red cells by quinine, the series of experiments recorded in Table 17 were carried out at temperatures of  $37^\circ \text{C}$ .,  $31^\circ \text{C}$ . and  $25^\circ \text{C}$ . respectively, the concentration of quinine (in the alkaloidal state) being 0.00113 M. (0.0366 per cent.). In order to avoid the effect of the diurnal variations in the composition of the red cells, illustrated in Table 16, each of the three sets of determinations (Experiments 1 to 4, Table 17) was carried at the same time with portions of the same emulsion of red cells in quinine solution. In Table 18, with the aid of the values of  $K$  obtained from Table 17, the value of  $\mu$  was determined. Before proceeding to consider the value of the latter constant, however, a few remarks may be made upon the data obtained in the former Table.

In the first place, it is to be observed that the same initial delay and terminal acceleration of the process of haemolysis, which has already been noted at  $37^\circ \text{C}$ . in Table 16, occurs also at  $31^\circ \text{C}$ . and  $25^\circ \text{C}$ . When the concentration of the red cells has been reduced by haemolysis to about 80 per cent., the reaction proceeds at all three temperatures at a tolerably constant rate until the concentration of red cells reaches about 20 per cent. Between these limits the values of  $K$ , calculated for a monomolecular rate, do not exhibit any very great variation; when calculated for a bimolecular or multimolecular reaction rate, the variations obtained are sufficiently great to exclude

TABLE 17. Rate of haemolysis of red blood cells, at different temperatures, by a 0.0113 M (0.366 %) solution of quinine in the alkaloidal state.

No. of Experiment	$t$	Per-centage of haemoglobin in red cells	$T\ 37^{\circ}\text{C.}$ $\frac{1}{t_n + 1 - t_n} \log \frac{C_n + 1}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K	$t$	Per-centage of haemoglobin in red cells	$T\ 31^{\circ}\text{C.}$ $\frac{1}{t_n + 1 - t_n} \log \frac{C_n + 1}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K	$t$	Per-centage of haemoglobin in red cells	$T\ 25^{\circ}\text{C.}$ $\frac{1}{t_n + 1 - t_n} \log \frac{C_n + 1}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K
1	0 min.	100		(175) Value of K taken at 37 (Table 18)	0 min.	100*		(229) Value of K taken at 55 (Table 18)	0 min.	100		(189) Value of K taken at 72 (Table 18)
	90 "	41	0.0043	19	90 "	84.6	0.0008*	55	150 "	75.8	0.0021	49
	135 "	12.3	0.0116	(8)	135 "	55.7	0.0040	33	210 "	48.8	0.0032	33
	180 "	2*	0.0156*	(8)	180 "	35.1	0.0045	25	270 "	32.9	0.0028	27.5
2	0 min.	100		(119) Value of K taken at 45 (Table 18)	0 min.	100		(115) Value of K taken at 46 (Table 18)	0 min.	100		Value of K taken at 72 (Table 18)
	60 "	45.5	0.0057	28	90 "	46.5	0.0037	34	150 "	75.8	0.0021	49
	90 "	25	0.0087	(17)	120 "	32.5	0.0051	25	210 "	48.8	0.0032	33
	120 "	7*	0.0184*	(10)	150 "	10*	0.0170*	25	270 "	32.9	0.0028	27.5
3	0 min.	100		(280) Value of K taken at 84 (Table 18)	0 min.	100		(220) Value of K taken at 72 (Table 18)	0 min.	100*		Value of K taken at 72 (Table 18)
	30 "	93.3*	0.0011*	46	90 "	71.6	0.0016	49	150 "	75.8	0.0021	49
	60 "	89.3	0.0007*	25.5	120 "	52.2	0.0046	34	210 "	48.8	0.0032	33
	90 "	43.8	0.0102	(14)	150 "	34.4	0.0061	28	270 "	32.9	0.0028	27.5
4	0 min.	100		(222) Value of K taken at 54 (Table 18)	0 min.	100*		(292) Value of K taken at 72.5 (Table 18)	0 min.	100*		Value of K taken at 72.5 (Table 18)
	105 "	92.6*	0.00031	38	90 "	91.7*	0.0022	72.5	150 "	75.8	0.0021	49
	135 "	87.9*	0.00070	32	120 "	88.3*	0.0027	64.5	210 "	48.8	0.0032	33
	165 "	83.4*	0.00070	27	150 "	85.6*	0.0040	55	270 "	32.9	0.0028	27.5
5	0 min.	100		(112) Value of K taken at 45 (Table 18)	0 min.	100		(115) Value of K taken at 46 (Table 18)	0 min.	100		Value of K taken at 72 (Table 18)
	90 "	41	0.0043	19	90 "	84.6	0.0008*	55	150 "	75.8	0.0021	49
	135 "	12.3	0.0116	(8)	135 "	55.7	0.0040	33	210 "	48.8	0.0032	33
	180 "	2*	0.0156*	(8)	180 "	35.1	0.0045	25	270 "	32.9	0.0028	27.5

\* Values neglected in calculating the mean value of K.

these reaction rates. Above the upper limit the values of  $K$  for a monomolecular reaction rate become increasingly small, and below the lower limit they become increasingly high. The marked character of the initial retardation and final acceleration is also well illustrated when the percentages (enclosed in brackets in Table 17), lying without the limits just given, are calculated according to the mean values of  $K$  obtaining within these limits. Two interpretations may be placed upon the different course taken by the reaction at its commencement and its termination: it may be that the reaction rate is, in itself, complex in character, becoming simple and monomolecular in one stage of its course; or it may be that the physical conditions, under which the reaction takes place, are such as to influence its rate at the beginning and again towards the end of the haemolysis, but not to any considerable extent in the rest of its course. In favour of the latter interpretation is the fact that, on the one hand alkaloidal quinine is in colloidal solution, and, therefore, cannot enter into a chemical reaction so readily as if it were in true solution, while on the other hand the red cell is surrounded externally by a membrane, which may offer physical difficulties to the entrance of the haemolytic agent into its interior.

TABLE 18. Determination of value of  $\mu$  from experiments recorded in Table 17.

No. of Experiment.	Temperature of experiment	Value of $K$ determined by experiment (Table 17)	Value of $\mu$ calculated from formula $\frac{K_2}{K_1} = e^{\frac{\mu}{2} \left( \frac{\tau_2 - \tau_1}{\tau_2 \tau_1} \right)}$	Percentage of red cells at beginning of experiment determined by haemoglobinometer	Value of $K$ calculated from $\mu = 15,000$
1	37° C.	0.0078	$t_{37^\circ}$ and $t_{31^\circ}$ 14500	0.408	0.0074
"	31° C.	0.0043	$t_{37^\circ}$ and $t_{25^\circ}$ 11500	0.408	0.0046
"	25° C.	0.0032	$t_{31^\circ}$ and $t_{25^\circ}$ 8800	0.408	0.0028
			mean 11600		
2	37° C.	0.0071	$t_{37^\circ}$ and $t_{31^\circ}$ 15000	0.400	0.0071
"	31° C.	0.0044		0.400	0.0044
"	25° C.	—		0.400	0.0027
3	37° C.	0.0102	$t_{37^\circ}$ and $t_{31^\circ}$ 23000	0.540	0.0087
"	31° C.	0.0047	$t_{37^\circ}$ and $t_{25^\circ}$ 14000	0.540	0.0054
"	25° C.	0.0040	$t_{31^\circ}$ and $t_{25^\circ}$ 4000	0.540	0.0033
			mean 14000		
4	37° C.	0.0030	$t_{37^\circ}$ and $t_{31^\circ}$ 22000	0.528	0.0026
"	31° C.	0.0015	$t_{37^\circ}$ and $t_{25^\circ}$ 18000	0.528	0.0016
"	25° C.	0.00095	$t_{31^\circ}$ and $t_{25^\circ}$ 14000	0.528	0.0010
			mean 18000		



Presumably dependent on the same cause are the variations in the values of  $K$  observable in the individual experiments in Table 17. These are greater than those in Table 16, ranging at  $37^{\circ}\text{C.}$  between 0.0030 and 0.0102 (or, when recalculated from the mean value of  $\mu$ , between 0.0026 and 0.0087). Table 17 shows that, in each emulsion these individual variations characterise the reaction rate at all temperatures employed; thus if the rate is rapid at  $37^{\circ}\text{C.}$ , it is also rapid at  $31^{\circ}\text{C.}$  and at  $25^{\circ}\text{C.}$ , and if relatively slow at the first temperature it is also relatively slow at the lower temperatures. This relationship is seen at a glance in Table 19, which illustrates the necessity of making simultaneous observations of the reaction rate of the same emulsion at different temperatures in order to determine the value of  $\mu$ ; obviously values of  $K$  taken at random from different experiments are of no value for this purpose. It will be noticed also that, owing to the large number of observations which require to be made, and the rapidity with which reaction proceeds at  $37^{\circ}\text{C.}$ , some difficulty is experienced in obtaining a sufficient number of estimations lying between 80 per cent. and 20 per cent. at this temperature. The values lying beyond these limits, which are neglected in calculating the mean value of  $K$ , are indicated, in the Table, with an asterisk. In Table 18 the initial percentages of red cells in the different experiments are given; no definite relation between the concentration of red cells employed and the values of  $K$  or  $\mu$  is to be observed.

TABLE 19. Comparison of values of  $K$  at different temperatures.

T	No. of Experiment.				Calculated for $\mu = 15000$				Mean
	1	2	3	4	1	2	3	4	
$37^{\circ}\text{C.}$	0.0078	0.0071	0.0102	0.0030	0.0074	0.0071	0.0087	0.0026	0.0065
$31^{\circ}\text{C.}$	0.0043	0.0044	0.0049	0.0015	0.0046	0.0044	0.0054	0.0016	0.0040
$25^{\circ}\text{C.}$	0.0032	—	0.0040	0.00095	0.0028	0.0027	0.0033	0.0010	0.0024

In Table 18 the value of  $\mu$  is determined, separately for each experiment, from the data given in Table 17, by the aid of the formula given on p. 33. In each experiment three values of  $\mu$  are obtained, the mean of which is taken as the true value. Although the mean values show some variation, being highest in Experiment 4,



in which haemolysis proceeded relatively slowly, and low in Experiments 1 and 3, in which haemolysis was relatively rapid, yet these variations are inconsiderable, the values of  $\mu$  lying within a narrow range, the highest mean value being 18,000 and the lowest 11,600, the mean of all being 15,000. From the latter value of  $\mu$  the lowering of the temperature required to diminish the rate of reaction to one-half may be calculated; at 37° C. it amounts to 9° C. With the aid of this mean value of 15,000 the percentages in Experiments 1 to 4, Table 17, are re-calculated, the values of  $K$  thus obtained not differing greatly from those determined by experiment. The difficulties in the way of carrying out these experiments, it may be observed, are by no means inconsiderable.

Additional confirmation of the conclusion already reached (p. 31), that haemolysis produced by the action of quinine in the alkaloidal form resembles a chemical process, not a physical one, is thus afforded by the circumstance that approximately identical values of  $\mu$  are obtainable in the experiments at varying temperatures recorded in Table 18.

The fact that, in the heterogeneous system formed by red blood cells suspended in a hydrosol of alkaloidal quinine, haemolysis progresses, except at the beginning and end of the process, at a monomolecular rate, indicates that quinine behaves like a catalytic agent, its action being similar to a ferment action comparable, for example, to that of pepsin. Quinine bihydrochloride, hydrochloric acid and sodium hydrate presumably also act like catalytic agents. The resemblance of these haemolytic agents to pepsin is all the greater when it is borne in mind that the amount of red cells completely haemolysed in three hours at 37° C. is proportional to the square root of the concentrations employed, for precisely the same relationship holds, within certain limits, for pepsin.\* The value of  $\mu$  for the digestion of gelatine by pepsin was found by Sjöqvist to be 10,750, and for the digestion of egg-albumin by pepsin to be 15,570. These values are not far removed from those obtained for the haemolysis of red cells by quinine.

It is to be anticipated that, as quinine in the free state, in producing haemolysis of red blood cells, resembles a catalytic agent,

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\* Sjöqvist, *Scandin. Archiv. f. Physiologie*, 5 (1895).

it is not used up to any considerable extent in the process, and that its concentration is therefore little altered by the reaction taking place. To test this point the series of estimations recorded in Tables 20 and 21 were made. In these estimations a weighed quantity of quinine sulphate was dissolved in such an amount of 0.9 per cent. sodium chloride solution as to produce about the same concentration as that employed in the experiments given in Table 1. Red cells were then added. In the first four experiments the weight of red blood cells taken was comparable to that in Table 1, in the remaining experiments much larger amounts of red cells were employed. When it was judged that sufficient time had elapsed for the red cells to take up quinine, the amount of the latter still remaining in solution was determined.

*Method.* In these estimations healthy human blood was employed throughout, being withdrawn by means of a sterilised glass injection syringe, from the cephalic, median-cephalic or ulnar vein of the upper limb, which was bandaged close to the axilla so as to obstruct the venous circulation. The time occupied in withdrawing blood was from ten to thirty seconds, and the amount withdrawn ranged from 4 c.cm. to 27 c.cm. Without any delay the blood so obtained was discharged into a beaker containing a freshly prepared 1 per cent. solution of potassium oxalate, in the proportion of five parts of the former to one part of the latter, and the mixture at once centrifugalised. The red cells quickly subsided and the supernatant serum was pipetted off. The red cells were then added in measured amounts to a solution of quinine sulphate, the bulk of which was about 200 c.cm. The emulsion was kept in a stoppered glass bottle which was shaken every half hour for two hours, after which the red cells were allowed to subside at room temperature ( $17^{\circ}$  C. to  $19^{\circ}$  C.), subsidence being completed in sixteen to twenty-four hours. No haemolysis took place. As much as possible of the supernatant liquid, which was clear or, with the larger amounts of red cells, very faintly opalescent, was removed with the aid of a capillary siphon, and the amount of fluid still remaining, usually not exceeding 5 per cent. of the original amount, was measured. It will be noted that the quinine solution after the first two hours was not everywhere equally exposed to the further action of the red cells, these gradually

disappearing from the upper\* part of the liquid and becoming increasingly dense below.

The amount of quinine in the supernatant liquid so obtained was then estimated, and from this the total amount of quinine still remaining in solution determined. The method of estimation employed was that of Giemsa and Schaumann,\* slightly modified to suit the conditions under which our investigation was carried out.

To the supernatant liquid, which was contained in a stoppered glass bottle holding 300 c.cm., about 2 g. of solid potassium hydrate were added. When the potassium hydrate had dissolved, the liquid had in most cases become milky in aspect, though no precipitate was recognisable. 70 c.cm. of ether were now added, the milkiness at once disappearing. The mixture of ether and watery solution was then shaken for half an hour. If the ether did not separate out, a small amount of alcohol was added, when ready separation took place. The ether was then transferred to a flask, by means of a capillary siphon, the amount of the upper layer of ether left behind being one-tenth to one-twentieth. Ether was now added again in three successive amounts of 50 c.cm., and siphoned off as before, in each case after half an hour's shaking. It may here be mentioned that three extractions with ether as recommended by Giemsa and Schaumann were not always sufficient to remove the last traces of quinine. The ether was then distilled off and the residue dried in a water oven at 98° C. When quite dry the residue was taken up with chloroform. The chloroform extract was put up in a weighing bottle, allowed to evaporate in a water oven at 70° C., then kept at 120° C. for one hour and weighed. The residue thus obtained was glassy in appearance, and colourless or of a faint brownish tint. It was found necessary to re-distil the ether and chloroform, and to test for the presence of any non-volatile residue before use. In the hands of Giemsa and Schaumann† three estimations of 0.05 g., 0.1 g. and 0.02 g. of quinine respectively gave an experimental error of - 0.4 per cent., + 1.0 per cent. and - 1.2 per cent. In our estimations two control amounts of 0.1100 g. are

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\* *Pharmakalogische und chemisch-physikalische Studien über Chinin*, Arch. f. Schiffs- u. Tropenhygiene, 1907, Bd. XI, S. 7.

† *Loc. cit.* p. 18.



recorded with an experimental error of  $-2.6$  per cent. and  $-1.5$  per cent. respectively.

The observations recorded in Tables 20 and 21 show that under the conditions of experiment obtaining, which, as already mentioned, were similar to those in Table 1, namely with a quinine sulphate solution of about 0.05 per cent. concentration and with an amount of

TABLE 20. Determination of the amount of quinine withdrawn from solution by healthy human red blood cells. Duration of action of red blood cells on quinine sulphate dissolved in 0.9 per cent. solution of sodium chloride eighteen to twenty-four hours. Temperature of experiment,  $17^{\circ}$  C. to  $19^{\circ}$  C.

No. of Experiment	Amount of quinine sulphate taken	Equivalent amount of quinine	Concentration of quinine sulphate	Amount of quinine recovered	Percentage of quinine recovered	Weight of wet red cells taken	Weight of wet red cells : Weight of quinine sulphate
1	0.1263 g.	0.1098 g.	0.0473 g.	0.1050 g.	95.6 %	1.5 g.	12 : 1
2	0.1263 g.	0.1008 g.	0.0547 g.	0.1047 g.	95.4 %	1.8 g.	14 : 1
3	0.1263 g.	0.1098 g.	0.0522 g.	0.1071 g.	97.5 %	2.2 g.	17 : 1
4	0.1193 g.	0.1037 g.	0.0529 g.	0.1023 g.	98.8 %	2.6 g.	22 : 1
5	0.1263 g.	0.1098 g.	0.0541 g.	0.0985 g.	88.7 %	7.9 g.	63 : 1
6	0.1193 g.	0.1037 g.	0.0534 g.	0.0907 g.	87.7 %	10.7 g.	90 : 1
7	0.1189 g.	0.1032 g.	0.0571 g.	0.0808 g.	78.3 %	12.0 g.	101 : 1
8 control	0.1267 g.	0.1100 g.	0.0517 g.	0.1071 g.	97.4 %	—	—
9 control	0.1267 g.	0.1100 g.	0.0517 g.	0.1084 g.	98.5 %	—	—

red blood cells in such excess as to prevent any trace of haemolysis during the course of the experiment, quinine is withdrawn from solution by red cells. In the first four experiments the withdrawal of quinine from solution by the action of the red cells is not satisfactorily exhibited since the amount taken up lies too near the error of experiment. When, however, the weight of red cells, instead of being 1.5 g. to 2.6 g. [Experiments 1 to 4, Table 20] is from 7.9 g. to 12 g., then the amount of quinine withdrawn, though relatively small, lies altogether beyond the range of experimental error and becomes readily ascertainable, varying between 11 per cent. and 22 per cent. of the amount originally present (Table 23). It is thus clear that the red cells take up quinine, and the amount which is combined is found (Experiments 5, 6 and 7) to be about 0.14 per cent. If we assume that the amount of quinine taken up by varying amounts of red cells, when the final concentration of quinine in the



surrounding fluid is in all cases the same, is proportional to the weight of red cells present, then in a final concentration of 0.0404 per cent. the amount of quinine taken up by 2 g. of wet red cells would be about 3 per cent. of the amount originally present in solution, under the conditions of experiment obtaining in Table 20. In view of the results obtained in the comparative experiments recorded in Table 9, it may be doubted if this simple relation holds. It may be suggested that the quantity taken up by the smaller amounts of red cells would

TABLE 21. Determination of the percentage amount of quinine, contained in red blood cells in the experiments recorded in Table 20.

No. of Experiment	Concentration of quinine sulphate originally present	Weight of wet red cells: Weight of quinine present at beginning of experiment	Percentage of the quinine, originally present, which was withdrawn from solution by red cells	Percentage of quinine in wet red cells	Final concentration of quinine in liquid part of emulsion of red cells
1	0.0473 % = 0.0412 % quinine	13.7 : 1	(4.4 %)	(0.320 %)	0.0393 %
2	0.0547 % = 0.0475 % "	16.4 : 1	(4.6 %)	(0.284 %)	0.0453 %
3	0.0522 % = 0.0453 % "	20.0 : 1	(2.5 %)	(0.123 %)	0.0442 %
4	0.0529 % = 0.0460 % "	25.0 : 1	(1.2 %)	(0.054 %)	0.0455 %
5	0.0541 % = 0.0470 % "	89.7 : 1	11.3 %	0.143 %	0.0417 %
6	0.0534 % = 0.0464 % "	103.3 : 1	12.3 %	0.121 %	0.0407 %
7	0.0571 % = 0.0496 % "	116.3 : 1	21.7 %	0.187 %	0.0388 %
mean of	5, 6 and 7 = 0.0477 quinine			mean of 5, 6 and 7 = 0.137 %	mean of 5, 6 and 7 = 0.0404 %

probably be relatively greater than that taken up by the larger amounts in Tables 20 and 21. Unfortunately the determination of the amount taken up by small quantities of red cells, representing the small difference of two estimations, each subject to an error of experiment, which is not of negligible dimensions, cannot be carried out with sufficient accuracy to settle this point.

Returning now to the enquiry, which formed the starting point of the experiments in Tables 20 and 21, namely, whether quinine is used up to any considerable extent when acting upon red cells so as to cause haemolysis, we find that under the conditions obtaining in the experiments recorded in Table 1 the concentration of the quinine solution undergoes a diminution, but that the extent of the diminution is very small. The conclusion arrived at on p. 38 is therefore confirmed.

A further relation exists to which reference may be made. So long as the haemolysis of red cells proceeds at a monomolecular rate the amount of red cells haemolysed during any short period of time is closely proportional to the concentration of the red cells in the emulsion at the beginning of the period. This is seen to be the case for Table 15, in which the weight of red cells haemolysed each half hour is found to be, for the calculated amounts, almost exactly  $0.236$  of the concentration at the beginning of each period [thus  $91.2 - 69.5 = 21.7 = 91.2 \times 0.238$ , and so on].

7. *Action of quinine on healthy red cells in the living body.*

The experiments recorded in Table 4 show that quinine bihydrochloride scarcely has any action upon red blood cells until its concentration reaches  $0.5$  per cent. If the percentage of quinine salt falls below this amount extremely little change takes place on keeping three hours at  $37^{\circ}\text{C}$ . Assuming the amount of blood in the human body to be  $5,000$  c.cm., the amount of quinine bihydrochloride required to produce a concentration of  $0.5$  per cent. would be  $25$  grammes ( $387$  grains), that is  $0.3$  g. per kilo of body weight, if the quinine were introduced intravenously. Since a dose of this amount, which would be rapidly fatal, is far above the maximum amount taken

TABLE 22. Quinine in relation to blackwater fever.

Case	Amount of quinine taken	Relation to appearance of blackwater
A	8 grs. at mid-day, 8 grs. at 3 p.m.	Passed blackwater at 6 p.m.
B	Two 10-gr. doses in afternoon and evening.	Passed blackwater at 1 a.m.
C	(1) 10 grs. a day for two days.	Passed blackwater on the third day.
	(2) (A year later) 10 grs. at 6 a.m.	Passed blackwater at 7 or 8 a.m.
D	(1) 10 grs.	Passed blackwater soon afterwards.
	(2) 5 grs. hypodermically.	Passed blackwater one and a half hours later.
E	10 grs. in evening.	Passed blackwater next morning.
F	6 grs. before and 6 grs. after dinner.	Passed blackwater in night.
G	Three 5-gr. doses of quinine.	After the first dose passed brown urine.
H	5 grs. of quinine a day for two days.	Blackwater on the fifth day.
	Took no more medicine.	
I	10 grs. for two evenings.	Blackwater second night.
J	6 grs. hypodermically at 10 a.m.	Urine porter coloured at mid-day.
K	10 grs.	Blackwater next day.
L	35 grs. in 5-gr. doses in two days.	Blackwater on third day.
M	9 grs. on two successive mornings.	Urine dark on second morning, porter coloured same evening.

medicinally, and as, moreover, quinine is not stored up in the body as such,\* it follows that the maximum amount taken medicinally, rarely reaching 3 grammes (46·5 grains), cannot produce haemolysis of healthy red blood cells in the circulation. The possibility of the haemoglobinuria of blackwater fever being due to the direct action of quinine on healthy red cells in the blood stream is thus absolutely excluded. That some other explanation of the action of quinine in causing blackwater fever must be sought is evident also from the smallness of the dose which ordinarily is followed by blackwater. Thus in Table 22, in which thirteen attacks are recorded, five grains or more hypodermically and nine grains or more by the mouth were followed by blackwater fever. These may be taken as representative amounts required to produce blackwater. In animals the hypodermic administration of quinine fails to produce haemoglobinuria, as the experiments recorded in Table 23 show, even when the dose is relatively far higher than in Table 22.

TABLE 23. Haemoglobinuria in relation to quinine in animals.

No. of Experiment	Animal	Amount of quinine bihydrochloride injected per kilo of body weight	Result
1	Rabbit	0·13 g. intravenously	Death within an hour.
2	"	0·08 g. hypodermically	No haemoglobinuria. No ill effect.
3	"	0·16 g. hypodermically	No haemoglobinuria. No ill effect.
4	"	0·5 g. hypodermically	No haemoglobinuria. Convulsions at end of 1½ hours; death at end of 2 hours.
5	Dog	{ 0·25 g. hypodermically 0·25 g. hypodermically 4½ hours later	No haemoglobinuria. Unsteady after first injection; convulsions and death ten minutes after second injection.
6	"	0·15 g. hypodermically	No haemoglobinuria. No ill effect.
7	"	0·2 g. hypodermically	No haemoglobinuria; no haemoglobinaemia Death at end of one hour.

The fate of quinine in the body is only partially known. Kleine,† Mariani,‡ Schmitz§ and Giemsa and Schaumann¶ showed that the

\* Loc. cit.

† Über die Resorption von Chininsalzen, Zeitschr. . Hygiene u. Infektionskrankheiten. Bd. 38, S. 190.

‡ L'assorbimento e l'eliminazione della chinina e dei suoi sali. Atti della Società per gli studi della malaria, 1904.

§ Über die Ausscheidung des Chinins im menschlichen Harn, Archiv. f. experimentelle Pathologie und Pharmakologie, 1907.

¶ Loc. cit.

amount of quinine eliminated as such in the urine during the first twenty-four hours after administration is usually 15 per cent. to 26 per cent. of the amount administered. In the body only traces of quinine can be recognised. Giemsa and Schaumann\* found quinine in the blood in only one out of three cases after its administration, and in this the quinine, which was present in amount too small for estimation, was found in the plasma only, not in the red cells. It would therefore appear that the amount of quinine reaching the blood stream and able to act directly upon the red cells is extremely small.

(b) *The action of quinine on red cells during blackwater fever.*

In the preceding subsection the action of quinine upon healthy red blood cells has been studied in considerable detail, so that the behaviour towards quinine of red blood cells during blackwater fever may be the more readily compared with their behaviour in respect of this alkaloid during health.†

*Method.* The method of testing employed has been that used in Table I, and involves the withdrawal of about ten drops (0.5 c.cm.) of blood from the finger, the procedure adopted being as follows: The blood coming from the finger was allowed to fall drop by drop into a small collecting capsule of glass, containing a measured amount of a 1 per cent. solution of potassium oxalate. As soon as enough blood had been collected the volume of the mixture was measured. The object of these two measurements was to ascertain the percentage by volume of red cells, and also the haemoglobinometer reading of the undiluted blood. If, however, it is not desired to obtain these data, then the two measurements in question may be omitted. The next step was to make a haemocrit determination of the percentage by volume of red cells in the oxalated blood. As soon as this had been done a measured amount of the oxalated blood was taken up in a pipette and transferred to a centrifugal tube containing about 10 c.cm. of a 0.9 per cent. solution of sodium chloride. After

\* Loc. cit. p. 32.

† A. Murri, Sull' intossicazione da chinino, *Il Policlinico*, 1895, Sezione Medica, Vol. 2, p. 340, tested the action of quinine in varying dilutions on the red blood cells of a patient suffering from haemoglobinuric fever following quinine, and on healthy red blood cells during similar conditions, but no difference was observable in the two cases. No details are given (p. 349).



completely precipitating the red cells by centrifugalisation, the supernatant liquid was removed by means of a glass tube provided with a rubber teat and drawn out to a capillary end. To the red cells 0.9 per cent. sodium chloride solution was added in such amount as to produce a 2.5 per cent. emulsion of the red cells. A sample of this emulsion was then diluted with nineteen parts of distilled water and a haemoglobinometer reading made. A series of test tubes was next prepared to which the respective quantities of 0.9 per cent. sodium chloride solution and of 1.92 per cent. quinine bihydrochloride solution given in Table 24 were added. The requisite amounts of the 2.5 per cent. emulsion of red cells were added, and the tubes placed in an incubator kept at 37° C., being subsequently stirred up with a glass rod every fifteen minutes until the conclusion of the experiment at the end of three hours.

In those experiments in which complete haemolysis did not occur the degree of haemolysis was indicated by the expressions 'partial,' 'slight,' 'trace.'

In carrying out these experiments it must be borne in mind that the emulsion should contain a definite weight of red blood cells. It cannot be made up with a definite weight of blood, for such a procedure would give no clue as to its real composition in respect of red cells, since it has been found that in health slight, and in black-water fever and malaria considerable, variations in the relation which the volume of red cells bears to that of the plasma may occur. Nor is it of any practical utility for our purpose to determine the number of red cells per cubic millimetre of blood, since this also gives no measure of their percentage by weight. Nor again is it of advantage to know the red cell index unless the percentage by volume is also known, and when the latter has been determined the former is not required. On the other hand, in order that the conditions of experiment may be more fully defined, it is desirable that the determination of the concentration of red cells by weight in the emulsion employed should be supplemented by a determination of the percentage of haemoglobin given by the reading of a haemoglobinometer standardised as already described. When the two determinations are nearly the same, no difficulty is experienced in comparing the haemolysis produced by quinine with that obtaining in health. When, however, a marked variation occurs, as in Experiments 10, 14a and

TABLE 24. The haemolytic action of quinine bishydrochloride upon red blood cells during and after blackwater fever and malaria. Experiments conducted at 37° C. as in Table 1 (p. 15). Duration of experiment three hours.

COMPOSITION OF MIXTURE OF RED CELLS AND QUININE SOLUTION											
No. of Observation	Case of Blackwater Fever	Condition present	Haemo- globin in wet red cells : volume of same.	0.9% NaCl ... 1.92% Q. 2 HCl ... 2.5% emulsion of wet red cells Concentration of Q. 2 HCl ... Weight of wet red cells ... Weight of Q. 2 HCl ...	1.00	1.20	1.50	1.80	2.30	2.80	3.20 c.c.
					0.05	0.05	0.05	0.05	0.05	0.05	0.05
					0.20	0.25	0.325	0.40	0.525	0.65	0.75
					0.080	0.065	0.0535	0.045	0.038	0.029	0.024%
					5.2	6.5	8.5	10.4	13.7	16.9	18.2
					1	1	1	1	1	1	1
1	3	During haemoglobinuria ...	0.91		Complete	Complete	Complete	Complete (in 2½ hrs.)	—	—	—
2	"	At end of attack of haemoglobinuria ...	0.91		Complete	Complete	Complete	Complete	Almost complete	Trace	Nil
3	"	A week after the disappearance of blackwater	1.11		Complete	Complete	Complete	Trace	Nil	Nil	Nil
4	4	Two days after relapse of haemoglobinuria ...	0.95		Complete	—	Partial	Almost complete	Trace	—	—
5	5	At end of attack of haemoglobinuria ...	1.05		—	Complete	—	—	Partial	—	—
6	7	During blackwater ...	1.18		Complete	—	Complete (in 1½ hrs.)	—	Partial	—	—
7	7 <sup>a</sup>	During haemoglobinuria ...	1.17		Complete	Complete	Complete	Marked	Trace	Trace	—
8	8	During haemoglobinuria ...	—		—	Complete	Complete	Complete	Almost complete	Slight	Nil
9	"	At end of attack of haemoglobinuria ...	—		—	Complete	Complete	Complete	Slight	Trace	Nil
10	9	Four days after attack of haemoglobinuria had ceased	1.36		—	Complete	Complete	Complete	Trace	Trace	—
11	10	During haemoglobinuria ...	1.01		Complete	Complete	Complete	Complete	Trace	Nil	Nil
12	"	During relapse of haemoglobinuria ...	1.18		—	Complete	Complete	Marked	Trace	—	—
13	11	Towards close of attack of haemoglobinuria ...	—		—	Complete	Complete	Complete	Trace	Trace	—
14	"	Three days after attack of haemoglobinuria (during partial suppression) ...	—		Complete	Complete	Complete	Complete	Trace	Trace	—
15	14	Ten hours after end of attack of haemoglobinuria...	—		Complete	Complete	Complete	Complete	Trace	Nil	Nil
16	14 <sup>a</sup>	Towards close of attack of haemoglobinuria ...	1.37		—	Complete	Complete	Complete	Trace	Nil	—
17	15	During haemoglobinuria ...	—		—	Complete	Complete	Complete	Trace	Trace	—
18	16	At end of attack of haemoglobinuria ...	—		—	Complete	Complete	Complete	Trace	Trace	—
19	—	Malarial attack, temperature normal, parasites numerous ...	1.24		Complete	Complete	Complete	Slight	Trace	Nil	—
20	—	Malarial attack, temperature 103° (twenty-four hours later parasites few) ...	1.12		Complete	Complete	Complete	Slight	Trace	Nil	—

19, Table 24, in which the ratio of the two determinations is respectively 1.36 : 1, 1.37 : 1 and 1.24 : 1, then a close comparison becomes difficult, since it is no longer possible to state the ratio of

TABLE I. Haemolysis of red blood cells by quinine bihydrochloride, dissolved in 0.9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.				
	Quinine bihydrochloride 0.080 %	0.065 %	0.054 %	0.045 %	0.038 %
	Weight of wet red blood cells 5.2 Weight of quinine salt 1	6.5 1	36.5 1	10.4 1	13.7 1
1	Complete	Complete	Complete	Complete	Partial
2	Complete	Complete	Complete	Complete	Marked
3	Complete	Complete	Complete	Complete	Partial
4	Complete	Complete	Complete	Complete	Partial
5	Complete	Complete	Complete	Complete	Partial

the red cells to quinine so precisely as could be desired. It is therefore evident that an extremely close comparison of the action of quinine on red cells in health and in blackwater fever cannot be made. The series of increments adopted in Tables 1 and 24 appear to be the smallest which can conveniently be chosen. Blackwater fever patients are frequently anaemic, and it will be noticed that the haemoglobin of the red cells was generally altered in the direction of an increase in the amount per unit volume of red cells. In Experiment 4, Table 24, although the direct haemoglobinometer reading of the blood was 24, the normal reading being 100, this ratio was scarcely affected, being 0.95.

In Table 24 the result of eighteen observations of the haemolysis of red cells by quinine in blackwater fever, and two observations in malaria, is given. For the sake of easy reference, Table 1 is repeated. In the latter Table the transition point is well defined, and is seen to be reached with a concentration of 0.045 per cent. of quinine bihydrochloride and a ratio of red cells (by weight) to quinine bihydrochloride of 10.4 : 1. In Table 24 the same result is obtained in nearly every case, the exceptions being Experiments 3, 4, 12, 19 and 20, in all of which haemolysis proceeded at a slightly slower rate than usual. Since occasional variations are also met with under normal conditions, (cp. Tables 2, 3, 5, 6, 7, 9, 11), it follows that within the limits of



accuracy of the method employed no decided change in the susceptibility of the red cells to the action of quinine is recognisable.

The same conclusion also holds if the conditions present are examined individually. During the period of haemoglobinuria eight cases were examined (1, 6, 7, 8, 11, 12, 13, 17); in five of these haemolysis was complete in three hours, the concentration of quinine bihydrochloride being 0.045 per cent.; in two (7, 12) haemolysis proceeded somewhat more slowly; and in one (6) the number of observations was small, but the rate of haemolysis was probably the same as that of the first five. Of the nine cases of blackwater fever examined when haemoglobinuria had ceased (2, 3, 4, 5, 9, 10, 14, 15, 18) six followed the normal course, in two (3, 4) haemolysis proceeded somewhat more slowly than usual, and the remaining one appeared to have followed the usual course, though the number of observations is incomplete.

Two observations were made during a malarial attack (19, 20), one during the apyrexial interval and one during the attack, when the patient's temperature was 103° F. In both of these the haemolysis proceeded at a slightly slower rate than usual, being completed in three hours with a concentration of quinine bihydrochloride of 0.0535 per cent, instead of the more usual concentration of 0.045 per cent. It cannot, however, be inferred from these two observations that a slight diminution in the rate of haemolysis is a constant occurrence, since a similar range of variation may be met with under normal conditions, as already mentioned.

Most of the thirteen attacks of blackwater fever referred to in Table 24 occurred in individuals who were regarded as also affected with malaria, since in all more or less typical malarial attacks preceded the paroxysm of blackwater fever, as a study of the clinical histories given on pp. 176 to 246 will show. Such evidence is, with one exception however, clinical only. In all cases quinine had been administered, and examination of blood films made at the time of blackwater failed to reveal the presence of malarial parasites in any case. The absence during blackwater fever of any obvious alteration in the vulnerability of the red blood cells towards quinine lends, however, further support to the view that no marked change in this respect is to be anticipated in malaria.



The circumstance, brought out quite conclusively in Table 24, that no considerable change in the red blood cells as regards the action of quinine occurs during blackwater fever, is a point of considerable importance, for a moment's consideration will show that, since healthy red cells are not haemolysed by even the greatest concentrations of quinine which may conceivably be reached in the blood when quinine is taken in the doses ordinarily administered in malaria, namely, one-third of a gramme to one gramme (five to fifteen and a half grains), therefore, unless the sensibility of the red cells is enormously increased in blackwater fever, no haemolysis due to the action of quinine on red cells contained in the circulating blood can possibly take place. The circumstance that, in point of fact, no marked change does occur, is therefore conclusive evidence against the action of quinine in blackwater fever being in any way related to a greater vulnerability of the red blood cells taken as a whole. The observations given in Table 24 do not afford any information as to whether any very small fraction of the red blood cells—such as would, owing to its relative insignificance, fail to be recognised by the method employed—may not be unduly sensitive to the action of quinine. In the next sub-section, an observation on the action of quinine on red blood cells containing malarial parasites is recorded, but the further consideration of this problem, which depends for its solution partly upon a knowledge of the actual amounts of red blood cells destroyed during attacks of blackwater fever, will be more conveniently deferred for the present, and dealt with again in Section 5 (pp. 136 to 165).

(c) *The action of quinine on red blood cells affected with malarial parasites.*

In any attempt to elucidate the mechanism of blackwater fever, it is a matter of the first importance to ascertain whether red blood cells affected with malarial parasites are more readily haemolysed by quinine than are healthy red cells. In order to study this point, it is necessary to obtain blood which is relatively rich in intracorpuseular parasites. Nearly all the cases of malaria which came under our notice, however, had already been treated with quinine, and parasites were found in the blood films examined in very scanty numbers or

could not be detected after prolonged search. Such cases were, therefore, of no value in settling the enquiry in question. Only a single case occurred in which we were able to test the action of quinine on red cells containing malarial parasites. In this case, on which Observations 19 and 20, Table 24, were made, a count of the red cells showed that about 3 per cent. contained parasites.

*Method.* The action of quinine was studied in the manner employed in the observations recorded in Table 1. Blood obtained by pricking the finger was allowed to fall drop by drop into a glass capsule containing 0.15 c.cm. of a 1 per cent. solution of potassium oxalate until about 1 c.cm. had been collected, blood smears being made at the same time. The total volume of the oxalated blood was then measured, and a haemocrit estimation made. The red cells were separated by centrifugalisation, washed and then added to 0.9 per cent. solution of sodium chloride in amount required to produce a 2.5 per cent. emulsion. This was mixed with 1.92 per cent. solution of quinine bihydrochloride and 0.9 per cent. solution of sodium chloride in the amounts given in Table 24. The mixtures, after stirring with a capillary glass rod, were kept in a water bath at 37° C. for three hours, when smears were made from the red cells contained in the last two tubes. The supernatant liquid after centrifugalisation was found to be colourless in the one tube, and to be of an exceedingly slight reddish tint in the other.

The result was indecisive. At the close of the experiment the number of parasites contained in red blood cells was diminished, and those present did not stain well. In addition a few free parasites were seen. It would thus appear that the action of the quinine salt was directed towards the parasites, the staining reaction of which was in consequence altered, while many disappeared or became unrecognisable. Some of the red blood cells which contained parasites appeared, therefore, to have been completely destroyed by the action of the quinine salt, for no red blood cells could be seen, which exhibited a partial defect of substance. All the red blood cells showed, however, slightly defective staining reaction, attributable to the action of quinine.

Unfortunately, we were able to make only a single experiment. The problem at issue cannot be settled until a number of such

observations have been made. In the meantime, we have thought it worth while to refer to the line of experiment followed.

*(d) The action of urine on red blood cells and on dissolved haemoglobin.*

In studying the mechanism of blackwater fever, it is essential to ascertain how far the condition of the urine when voided represents its original condition when issuing from the collecting tubules of the kidney, and how far it has been changed while retained in the bladder. This enquiry resolves itself into a two-fold one: into the action of urine upon haemoglobin contained in the red blood cells; and into the action of urine upon laked haemoglobin.

When a solution of haemoglobin is added to urine in amount sufficient to produce a light red coloration, it is found that the red tint soon becomes fainter, the urine turning brown in colour, and that ultimately the whole of the haemoglobin is broken up, a brown soluble pigment remaining, which gives no absorption bands on spectroscopic examination. Sometimes a variable amount of a brown precipitate appears at the end of twenty-four to forty-eight hours, and the urine may then become less brown in colour.

If an emulsion of red blood cells in 0.9 per cent. solution of sodium chloride is added to urine, it is seen that the latter, when the changes in the red cells described below, become advanced, gradually turns brown in colour, but no reddish tint is noticeable. Urine does not produce visible laking of red blood cells unless its specific gravity is less than 1.009. Urine of specific gravity 1.002 laves red blood cells as rapidly as distilled water. Red blood cells in urine whose specific gravity exceeds 1.009 become, on standing, poorer in haemoglobin and assume a darker somewhat brownish aspect, but are ultimately decolourised and form a brownish-white precipitate at the bottom of the tube in which the urine is contained. If such urine at any time after adding the red blood cells is centrifugalised and the supernatant fluid examined spectroscopically, it is found that no oxyhaemoglobin bands are at first seen, the red blood cells becoming decolourised without any laking of their haemoglobin. When, however, the red blood cells have lost 75 per cent. or more of their haemoglobin, oxyhaemoglobin bands make their appearance in the urine, which is now



brownish in colour (cp. Table 23). Just as occurs when dissolved haemoglobin is added to urine, a precipitate brown in colour may make its appearance at the end of twenty-four hours in the mixture of urine and red cells. It is thus seen that in the red cell the haemoglobin is broken up, by urine of specific gravity exceeding 1.009, *in situ*, and does not leave the cell until it has become completely converted into brown pigment.

In order that some idea may be obtained of the degree to which haemoglobin is likely to be broken up in the urine in cases of black-water fever, and, further, in order to determine to what extent small quantities of haemoglobin passing into the urine at the time of its secretion by the kidneys may be recognisable in the urine when voided, the experiments given in Table 25 were carried out. These fall into two series, conducted simultaneously with the same urine and with haemoglobin derived from a single source.

*Method.* In the first series of experiments human blood cells were added to the urine in such amounts that an emulsion containing from 5 per cent. to 0.33 per cent. of red blood cells was obtained. At the end of four hours at a temperature of 37° C., during which period the red blood cells were distributed in the urine by stirring with a glass rod every fifteen minutes, the mixture was centrifuged, the supernatant fluid poured off and examined spectroscopically, while the red cells were mixed with a measured quantity of distilled water and, after laking, the amount of haemoglobin still remaining determined by the haemoglobinometer. In the second series the same amounts of haemoglobin were used as in the first, but the haemoglobin instead of being contained in red cells was in solution. In this second series, owing to the formation, especially in the weaker dilutions, of the brownish pigment already referred to, accurate haemoglobinometer readings of the amounts of haemoglobin in the urine could not be made, and instead the oxyhaemoglobin bands present were matched by the aid of a comparison spectroscope with those of a haemoglobin solution of known concentration, and in this way the concentration of haemoglobin in the urine was determined.

On comparing the two series, it is seen that the degree of destruction of haemoglobin proceeds in both very nearly at the same rate, being somewhat more rapid when the haemoglobin is in solution than when it is contained in the red blood cells, but the difference is



TABLE 25. The action of urine in breaking up haemoglobin. Duration of experiment four hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF URINE AND RED CELLS						COMPOSITION OF MIXTURE OF URINE AND DISSOLVED HAEMOGLOBIN						CONTROL TUBE	REMARKS
	Amount of urine ...	0.80 c.c.	1.80 c.c.	4.80 c.c.	9.80 c.c.	14.80 c.c.	Amount of urine ...	0.80 c.c.	1.80 c.c.	4.80 c.c.	9.80 c.c.	14.80 c.c.		
	Amount of red cells ...	0.05 c.c.	0.05 c.c.	0.05 c.c.	0.05 c.c.	0.05 c.c.	Amount of dissolved haemoglobin ...	0.05 c.c.	0.05 c.c.	0.05 c.c.	0.05 c.c.	0.05 c.c.	0.05 c.c.	
	Amount of 0.9 % NaCl solution ...	0.15 c.c.	0.15 c.c.	0.15 c.c.	0.15 c.c.	0.15 c.c.	Amount of 0.9 % NaCl solution ...	0.15 c.c.	0.15 c.c.	0.15 c.c.	0.15 c.c.	0.15 c.c.	0.15 c.c.	
	Concentration of urine in mixture ...	80 %	90 %	96 %	98 %	98.7 %	Concentration of urine in mixture ...	80 %	90 %	96 %	98 %	98.7 %	98.7 %	
	Weight of wet red cells	1	1	1	1	1	Weight of haemoglobin	1	1	1	1	1	1	
	Weight of urine ...	16	36	96	196	294	Weight of urine ...	16	36	96	196	294	294	
1	Percentage of wet red cells ...	5 %	2.5 %	1 %	0.5 %	0.33 %	Percentage of haemoglobin ...	5 %	2.5 %	1 %	0.5 %	0.33 %	0.33 %	Sp. gr. of urine 1.025. Acid. No albumin.
	Amount of haemoglobin remaining in red cells at end of experiment	65 %	51.5 %	33 %	22.5 %	Too small for measurement	Amount of haemoglobin remaining at the end of experiment	—	—	—	—	—	0.524 %	
	Amount of haemoglobin destroyed ...	35 %	48.5 %	67 %	77.5 %	—	Amount of haemoglobin destroyed ...	—	—	—	—	—	—	
	Amount of haemoglobin remaining in red cells at end of experiment	52.5 %	39 %	32 %	—	—	Amount of haemoglobin remaining at the end of experiment	—	37 %	32 %	—	—	0.556 %	
	Amount of haemoglobin destroyed ...	47.5 %	61 %	68 %	—	—	Amount of haemoglobin destroyed ...	—	63 %	68 %	—	—	—	
2	Amount of haemoglobin remaining in red cells at end of experiment	60 %	42 %	39 %	29 %	11 %	Amount of haemoglobin remaining at the end of experiment	42 %	36 %	28 %	26 %	11 %	0.556 %	Sp. gr. of urine 1.018. Acid. No albumin.
	Amount of haemoglobin destroyed ...	40 %	58 %	61 %	71 %	89 %	Amount of haemoglobin destroyed ...	58 %	64 %	72 %	74 %	89 %	—	
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	
3	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	Sp. gr. of urine 1.020. Acid. No albumin.
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	
4	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	Sp. gr. of urine 1.015. Faintly Acid. No albumin.
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	
	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment	51 %	51 %	42 %	29 %	13 %	—	

not great. As might be expected, the destruction is relatively smallest when the concentration of the red blood cells, or of the laked haemoglobin, is greatest, and when the concentration of haemoglobin is very low the destruction may be very nearly complete within the period of experiment. The actual figures given will enable some idea to be formed of the degree to which in blackwater fever the amount of haemoglobin present at the end of four hours represents the amount originally present in the urine when first excreted. Thus when the urine originally contained 5 per cent. of red blood cells, the percentage at the end of four hours was only 2·7 per cent. to 3·25 per cent., 35 per cent. to 47 per cent. of the haemoglobin being destroyed. When, however, the urine originally contained 0·33 per cent. of haemoglobin, about 90 per cent. was destroyed, the percentage being reduced to 0·03, a quantity which is not easily recognised or estimated. The percentages in the Table were for the most part ascertained by means of haemoglobinometer readings. The original concentrations were made up by means of the haemocrit, and one of these, as a control, was measured by the haemoglobinometer, the reading obtained being a little higher (0·5 per cent.) than the amount given by the haemocrit.

When the percentage of haemoglobin, whether in the form of red blood cells or of dissolved haemoglobin, was less than 0·01 per cent. in the urine, at the end of four hours it became difficult or impossible to recognise oxyhaemoglobin bands. In these cases the urine, which, as already mentioned, had acquired a brownish tint, gave, on slightly acidifying and heating, a brownish white precipitate of coagulable protein, this forming in many cases the only indication of the original addition of haemoglobin.

Owing to the circumstance that urine is not a fluid of constant composition, it is not possible to calculate, from the amount present at the end of four hours, how much was originally present. In each individual case a special estimation is required. Only an approximate idea of the limits of the range of destruction in different concentrations is afforded by Table 25, which, however, shows that with a high specific gravity of the urine the rate at which destruction proceeds is increased. Destruction of haemoglobin was usually attended with the production of methaemoglobin, which was, however, present only

in relatively very small amount. This occurred both within the red cells, and when dissolved haemoglobin was used.

With a view of throwing light upon the nature of the process of destruction of haemoglobin by urine, the rate at which destruction proceeded was investigated in the case of red cells (Table 26). The determination of the rate of destruction, in urine, of dissolved haemoglobin was not attempted owing to the difficulty of obtaining, especially with the higher degrees of destruction, sufficiently readily and accurately estimations of haemoglobin in the presence of the colouring matter of urine.

*Method.* The mode of conducting these experiments was similar to that adopted in the case of quinine (Tables 17, 18 and 19). Four experiments were carried out with different specimens of healthy urine obtained from the same individual, about three hundred cubic centimetres being taken for each experiment. To this, healthy human red blood cells were added to the extent of 0.488 per cent., 0.484 per cent., 0.448 per cent. and 0.488 per cent. in Experiments 1 to 4 respectively. The mixture of urine and red blood cells was then divided into three equal parts, which were placed in water baths kept at temperatures of 37° C., 31° C. and 25° C. respectively, and were shaken every fifteen minutes so as to secure uniform distribution of the red blood cells. At intervals of half to one hour samples of the mixture (usually 10 c.cm.) were withdrawn and centrifugalised. The supernatant liquid was examined for oxyhaemoglobin and methaemoglobin bands; the red cells were laked with distilled water, the volume of the solution being made up to that of the sample and the percentage of haemoglobin determined with the haemoglobino-meter or, if considerable destruction of haemoglobin and production of brown colouring matter had occurred, matched with a haemoglobin solution of known concentration, by the aid of a comparison spectroscope, as already described.

The urine employed in these four experiments varied in respect of specific gravity and reaction (cp. Table 27). The former ranged from 1.026 to 1.015, while the reaction was acid in Experiment 1, slightly acid in Experiments 2 and 3, and slightly alkaline in the fourth experiment. The urine was perfectly clear and free from precipitate.



TABLE 26. The rate of destruction of haemoglobin in red blood cells by urine at different temperatures.

No. of Experiment	$t$	Percentage of haemoglobin in red cells	$T\ 37^{\circ}\text{C.}$ $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K	$t$	Percentage of haemoglobin in red cells	$T\ 31^{\circ}\text{C.}$ $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K	$t$	Percentage of haemoglobin in red cells	$T\ 25^{\circ}\text{C.}$ $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K
1	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	90 "	41	0.0043	41 taken at	90 "	72	0.0016	70	120 "	90	0.00038*	85
	150 "	12.5**	0.0060	24 0.0044	150 "	55	0.0017	55	180 "	71	0.00081	78 Value of K
	210 "	1**	0.0096*	12 (Table 24)	210 "	48	0.0015	44	240 "	60	0.00093	72 taken at
					285 "	37	0.0015	33	285 "	58	0.00082	67 0.0006
			0.0051 mean		345 "	25	0.0017	26	345 "	54	0.00078	63 (Table 24)
2	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	30 "	90	0.0015*	85 taken at	30 "	99	0.0001*	93	165 "	91	0.00035*	87 Value of K
	60 "	81	0.0015	70 Value of K	60 "	97	0.0002*	86	225 "	86	0.00031	83 taken at
	90 "	66	0.0017	61 taken at	90 "	88	0.0005*	80	285 "	81	0.00035	79 0.00037
	150 "	45	0.0022	44 0.0024	120 "	82	0.0007	76	345 "	71	0.00043	74 (Table 24)
	180 "	38	0.0023	37 (Table 24)	150 "	74	0.0009	69	405 "	67	0.00043	71
3	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	30 "	90	0.0015*	85 taken at	30 "	99	0.0001*	93	165 "	91	0.00035*	87 Value of K
	60 "	81	0.0015	70 Value of K	60 "	97	0.0002*	86	225 "	86	0.00031	83 taken at
	90 "	66	0.0017	61 taken at	90 "	88	0.0005*	80	285 "	81	0.00035	79 0.00037
	150 "	45	0.0022	44 0.0024	120 "	82	0.0007	76	345 "	71	0.00043	74 (Table 24)
	180 "	38	0.0023	37 (Table 24)	150 "	74	0.0009	69	405 "	67	0.00043	71
4	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	30 "	90	0.0015*	85 taken at	30 "	99	0.0001*	93	165 "	91	0.00035*	87 Value of K
	60 "	81	0.0015	70 Value of K	60 "	97	0.0002*	86	225 "	86	0.00031	83 taken at
	90 "	66	0.0017	61 taken at	90 "	88	0.0005*	80	285 "	81	0.00035	79 0.00037
	150 "	45	0.0022	44 0.0024	120 "	82	0.0007	76	345 "	71	0.00043	74 (Table 24)
	180 "	38	0.0023	37 (Table 24)	150 "	74	0.0009	69	405 "	67	0.00043	71

\* Values neglected in calculating mean value of K. \*\* Haemoglobin present in solution in urine; these values are consequently too low.



The destruction of haemoglobin proceeded rapidly at  $37^{\circ}\text{C}$ ., usually half to three-quarters disappearing in the course of two hours. At  $31^{\circ}\text{C}$ . one-fifth to two-fifths of the haemoglobin was destroyed in the same period of time, and at  $25^{\circ}\text{C}$ . less than one-tenth. When a comparison of these amounts with the specific gravity and reaction of the urine is made (Table 25), it is seen that with the higher specific gravity (1.026) obtaining in Experiment 1 the rate of destruction was most rapid, with the lower specific gravity of Experiments 2 and 3 (1.020 and 1.022) was less rapid, while in Experiment 4 in which the urine had a specific gravity of 1.015, haemoglobin was destroyed with considerable slowness. In the last case another factor was perhaps operating to retard the action of the urine upon haemoglobin, namely, its alkaline reaction; for alkaline urine was found to be slow in its action upon red cells, even when its specific gravity exceeded 1.020. The variations met with in different experiments seem, however, to be in part attributable to the diurnal variations in the red blood cells already encountered in experiments made with quinine (Tables 16 and 17). The breaking up of haemoglobin in the urine is not due to the presence in the latter of a thermolabile ferment, for previous boiling does not alter the rate at which destruction proceeds.

In red cells, whose haemoglobin has been largely destroyed, methaemoglobin is found, on laking, to be present. The amount of this substance is very small relatively to the unaltered haemoglobin, until the destruction exceeds 85 per cent., when its presence is generally readily recognisable, though it is still found in much smaller amount than oxyhaemoglobin.

It will be seen from Table 26 that haemoglobin did not find its way into the urine until the destruction of haemoglobin within the red cells had reached or exceeded 75 per cent. At about this point haemoglobin began to escape, and when the percentage of destruction exceeded 90 per cent., the percentage of haemoglobin in the urine was sometimes 5 per cent. of the amount originally present in the red blood cells, in one case reaching as much as 10 per cent. Thus, although haemolysis did not occur in amount sufficient to produce after centrifugalisation a red coloration of the urine, nevertheless as destruction of haemoglobin approached completion a slight degree of haemolysis took place, causing oxyhaemoglobin bands to make their appearance.

In determining the reaction rate it will be seen that at the highest temperature employed ( $37^{\circ}\text{C.}$ ) the number of observations obtainable in Experiments 1 and 3, Table 26, was limited, owing partly to the rapidity of destruction of haemoglobin and partly to the escape of haemoglobin from the red cells into the urine surrounding them, in consequence of which the readings given by the laked cells become too low, and cease to represent the actual destruction. In Experiments 1 to 3, determinations of the monomolecular reaction rate constant show little variation so long as the amount of destruction of haemoglobin lies between 20 per cent. and 80 per cent. Below the lower limit destruction proceeds more slowly, while above the upper limit it proceeds more rapidly. The same variation, in greater degree, has already been seen when quinine produces haemolysis in red blood cells (Tables 13 to 16). And as in the latter case, so here, if the reaction rate constant for bimolecular or multimolecular reactions is calculated from the data given, a greater range of variation is met with, so that these types of reaction are excluded from consideration. In Experiment 4, in which the reaction proceeded very slowly, the reaction rate constant exhibited little variation when the destruction exceeded 7 per cent. (93 per cent. being left unaltered), i.e. after the lapse of about two hours. If the percentages of haemoglobin are recalculated from the reaction rate constants (Columns 4, 8 and 12, Table 24), a fair agreement with the percentages actually found will be observed. It follows therefore that, in the heterogeneous system formed by red blood cells suspended in urine, destruction of haemoglobin proceeds at the same rate as a monomolecular reaction, except at the beginning and end of the process, when slight delay and acceleration respectively occur.

The values of  $K$ , the monomolecular reaction rate constant, obtained with the different specimens of urine employed, range from 0.0003 to 0.005 at  $37^{\circ}\text{C.}$ , from 0.0002 to 0.002 at  $31^{\circ}\text{C.}$ , and from 0.0008 to 0.0001 at  $25^{\circ}\text{C.}$  The values obtained in urine of specific gravity 1.015, whose reaction to litmus paper was alkaline, are, it will be noted, much lower than those obtained in acid urine of higher specific gravity.

In Table 27 the value of the constant  $\mu$  was determined for each of the four specimens of urine employed. The values obtained,

ranging from 13,000 to 30,000, increased as the specific gravity increased and the reaction changed from alkaline to acid (to litmus paper). In the last column the values of  $K$  are recalculated from the value of  $\mu$  obtained and from the mean value of  $K$  at  $31^{\circ}\text{C}.$ ; a fair agreement is observable between the values found and calculated.

TABLE 27. Determination of value of  $\mu$  from experiments recorded in Table 26.

No. of Experiment	Temperature of experiment	Value of $K$ determined by experiment (Table 26)	Value of $\mu$ calculated from formula	Condition of urine	Value of $K$ calculated from $\mu$
			$\frac{K_2}{K_1} = e^{\frac{\mu}{2} \left( \frac{\tau_2 - \tau_1}{\tau_2 \tau_1} \right)}$		
1	$37^{\circ}\text{C}.$	0.0051	30,000	Acid; sp. gr. 1.026	0.0044
"	$31^{\circ}\text{C}.$	0.0017			0.0017
"	$25^{\circ}\text{C}.$	0.0008			0.0006
2	$37^{\circ}\text{C}.$	0.0021	24,100	Slightly acid; sp. gr. 1.020	0.0024
"	$31^{\circ}\text{C}.$	0.0011			0.0011
"	$25^{\circ}\text{C}.$	0.00038			0.00037
3	$37^{\circ}\text{C}.$	0.0043	19,400	Slightly acid; sp. gr. 1.022	0.0039
"	$31^{\circ}\text{C}.$	0.0021			0.0021
"	$25^{\circ}\text{C}.$	0.00079			0.00088
4	$37^{\circ}\text{C}.$	0.00032	13,600	Alkaline; sp. gr. 1.015	0.00026
"	$31^{\circ}\text{C}.$	0.00017			0.00017
"	$25^{\circ}\text{C}.$	0.00013			0.000092

In Table 26 it will be noticed that the percentages of haemoglobin, remaining undestroyed, have been recalculated in columns 5, 9 and 13, from the corresponding values of  $\mu$ , taken from the last column of Table 27. The difference between the observed and calculated percentages is seen to be inconsiderable, except when destruction of haemoglobin exceeds 80 per cent.; in the latter case the percentages cannot be estimated with much precision owing to the escape of haemoglobin from the red cells. Table 28 is introduced in order to permit a more ready comparison of the values of  $K$ , found and calculated, at individual temperatures. The values of  $\mu$ , obtained in Table 27, enable the lowering of the rate of destruction of haemoglobin, caused by variation of temperature, to be calculated. Thus a reduction of the destruction rate to  $\frac{1}{2.163}$  takes place if the temperature is lowered from  $37^{\circ}\text{C}.$  to  $31^{\circ}\text{C}.$  when  $\mu = 30,000$  and to  $\frac{1}{1.54}$

when  $\mu = 13,600$ ; in the former case a reduction of the temperature of  $37^{\circ}\text{C}$ . by one degree leads to a reduction of the rate of destruction from 1 to 0.855, and in the latter case from 1 to 0.925. The same relationship may be stated in another way: the rate of destruction of haemoglobin at  $37^{\circ}\text{C}$ . becomes reduced to one half when the temperature is lowered  $5^{\circ}\text{C}$ . if  $\mu = 30,000$ , and when the temperature is lowered  $9^{\circ}\text{C}$ . if  $\mu = 13,600$ . These values are nearly the same as those obtaining in the actual experiments.

TABLE 28. Comparison of values of K at different temperatures. Cp. Table 27.

T	No. of Experiment				Calculated for			
					$\mu = 30,000$	24,100	19,400	13,600
	1	2	3	4	1	2	3	4
$37^{\circ}\text{C}$ .	0.0051	0.0021	0.0043	0.00032	0.0044	0.0024	0.0039	0.00026
$31^{\circ}\text{C}$ .	0.0017	0.0011	0.0021	0.00017	0.0017	0.0011	0.0021	0.00017
$25^{\circ}\text{C}$ .	0.0008	0.00038	0.00079	0.00013	0.0006	0.00037	0.00088	0.000092

It is thus seen that in the presence of urine the rate of destruction of haemoglobin contained in red blood cells resembles that of a monomolecular process, and is in this respect similar to the rate of haemolysis of red blood cells in the presence of quinine in the alkaloidal state (Tables 13 to 19). The two processes are, however, of a different character, the former being apparently a direct action upon haemoglobin, while the latter seems to be the result of an action directed primarily to the stromata of the red blood cells.

### SUMMARY

The principal points in the above investigation may be summarised as follows:—

1. Quinine bihydrochloride and quinine in the alkaloidal state produce haemolysis of red blood cells, as do also hydrochloric acid and sodium hydrate.
2. The action of quinine in the alkaloidal state in producing haemolysis resembles a catalytic action.



3. Haemoglobin breaks up at a monomolecular rate under the action of quinine in the alkaloidal state.

4. The above-mentioned four haemolytic agents possess, in equimolecular concentration, nearly the same haemolytic power, quinine in the alkaloidal state being weaker, and quinine bihydrochloride stronger than hydrochloric acid and sodium hydrate, which occupy an intermediate position.

5. Owing to the toxicity of quinine its concentration in the blood cannot reach an amount sufficient to allow of its direct haemolytic action on red cells taking place during life.

6. The red blood cells during blackwater fever are not haemolysed by quinine bihydrochloride more readily than in health.

7. In the presence of urine haemoglobin, whether contained in red blood cells or in solution, is broken up. In the former case this proceeds at a monomolecular rate, no haemoglobin being discharged from the red cells into the urine, until destruction is nearly complete.

8. The constant,  $\mu$ , for the haemolysis of red blood cells was found to have a mean value of 15,000 for a 0.00113 M solution of alkaloidal quinine. The value of  $\mu$  for the destruction of haemoglobin in the presence of urine ranged, in the experiments made, from 13,000 to 30,000. The values of  $K_{37^\circ}$  ranged in the former cases from 0.0026 to 0.0087, and in the latter case from 0.0026 to 0.0039.

## II. THE RELATION OF HAEMOLYSINAEMIA TO THE HAEMOGLOBINURIA OF BLACKWATER FEVER.

In no single instance, up to the present time, has the mechanism of production of haemoglobinuria in blackwater fever been established, though assertions respecting its mode of production are constantly to be met with in text-books and in clinical articles. The only form of haemoglobinuria, of which the mechanism is in part known, is paroxysmal haemoglobinuria, and before proceeding further it will be of advantage briefly to recapitulate the facts which have recently been established in connection with the mechanism of production of this condition. In 1904 Donath and Landsteiner\* showed that, in the intervals between the attacks of haemoglobinuria, if the blood rendered fluid by potassium oxalate, or a mixture of the patient's serum and the patient's washed red blood cells, was cooled and then warmed to body temperature, a marked haemolysis occurred, which was not observed if the blood was kept at body temperature, without previous cooling. The red blood cells of the patient were found to be normal, and might be replaced by foreign human red cells; only the serum or plasma is changed, and the haemolysin could be extracted in the cold from the serum by red blood cells, which became dissolved when the serum of the patient or normal human serum was added, the reaction being completed by the addition of complement. The minimum time of cooling required in ice cold water was five to ten minutes (in one case two minutes). Cooling to 10° C. for half an hour was followed by partial haemolysis. This condition was not met with in health, nor in patients suffering from any disease other than paroxysmal haemoglobinuria, with the exception of general paralysis, in which affection it was found that in six out of sixty-six cases the same reaction was obtained.

Donath's and Landsteiner's results were confirmed and their conclusions disputed by Widal and Rostaine,† who have arrived at a somewhat different interpretation of the reaction in question. These

\* Über paroxysmale Hämoglobinurie. Münch. med. Wochenschr., 1904, No. 36.; Über paroxysmale Hämoglobinurie, Zeitschr. f. klin. Medizin, 1906, Bd. 58, S. 173.

† Insuffisance de l'antisensibilisatrice dans le sang des haemoglobinuriques. Comptes rendus de la Société de Biologie, 1905, T. 1, p. 321; Insuffisance de l'antisensibilisatrice dans le sang d'un haemoglobinurique, *ibid.*, p. 372; Sérothérapie préventive de l'attaque de l'hémoglobinurie paroxystique, *ibid.*, p. 397.

authors point out that Bordet had demonstrated the existence of 'substance antisensibilisatrice' (anti-immune body) for red blood cells, and Metchnikoff for spermatoxine, while Besredka has shown that red blood cells remain preserved in their own serum in consequence of the inhibitory action of the substance 'antisensibilisatrice' upon the 'sensibilisatrice' usually present. The authors have come to the conclusion that the haemolysis in vitro obtained by Donath and Landsteiner is due to the insufficiency of the substance 'antisensibilisatrice.' They appear to base their conclusion upon the effect of an 'antisensibilisatrice' serum, which they prepared, one drop of which inactivated ten drops of the plasma of a patient suffering from paroxysmal haemoglobinuria, to which three drops of human red cells were added. The antiserum was prepared by injecting, three or four times, massive doses of the serum of a haemoglobinuric patient into animals. The authors used this serum with complete success to arrest haemoglobinuria when cold was applied to the patient's hands, but with only partial success when the test was made in vitro.

In view of the above facts, we decided to ascertain if the blood plasma in blackwater fever possessed a similar haemolytic property.

*Method.* Our own experiments were conducted as follows:—The patient's finger, having been cleansed, was pricked and about ten drops (0.5 c.cm.) of blood were allowed to flow into a small collecting tube containing 1 c.cm. of a 1 per cent. solution of potassium oxalate. The oxalated blood was then transferred by means of a fine pointed pipette to a small centrifugal tube. After centrifugalisation the plasma was pipetted off and put into another tube. The red cells were then made up into a 2.5 per cent. emulsion in 0.9 per cent. sodium chloride solution, and of this one part was added to nineteen parts of the oxalated plasma, so that the ratio of the weights of the wet red cells and plasma respectively would be one to seven hundred and fifty. In some of the experiments, as Tables 29 and 30 show, human red cells not taken from the patient were employed. In some of the later experiments also red blood cells were added in such an amount as to produce a thin emulsion, without reference to the exact proportion of red cells present. The tube containing the plasma and red cells was then put into a freezing mixture obtained by adding solid ammonium nitrate to water contained in a beaker,

which was placed in a second larger beaker, surrounded by cotton wool and supported on cork. Between the two beakers an air space existed, as shown in Fig. 1. A thermometer dipped into the ammonium nitrate mixture was used for stirring up the solid salt from time to time as required. In this way the temperature of the mixed plasma and red cells was kept at  $0^{\circ}\text{C}$ . to  $5^{\circ}\text{C}$ . If desired a temperature below  $0^{\circ}\text{C}$ . could have been maintained. The time elapsing between collecting and centrifugalising the blood was in most cases about six minutes; that between pipetting off the plasma and cooling it in the freezing mixture was generally about twenty minutes to two hours. While in the freezing mixture the red cells were once or twice distributed in the plasma by stirring with a capillary glass rod. After being kept in the freezing mixture for half an hour, the tube containing the plasma and red cells was put for three hours in an incubator kept at  $37^{\circ}\text{C}$ ., the red cells being stirred from time to time with a glass rod as before.

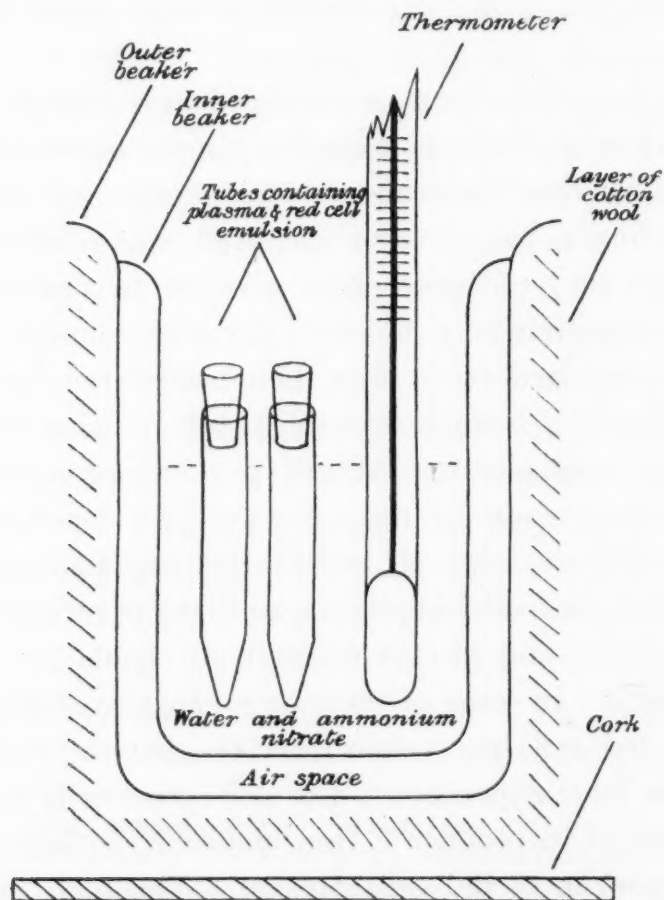


FIG. 1. Apparatus employed for testing haemolytic property of blood plasma.



TABLE 29. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from blackwater fever.

No. of Observation	Case	Condition of subject	Ratio of oxalated plasma to 2.5 % emulsion of red cells	Mixture kept at	For	Then kept at 37° C. for	Result
1	2	Sixteen hours before attack of blackwater	20 : 1	0°C.-4°C.	30 min.	3 hours	No isolysin present in blood plasma
2	"	Two hours before attack of blackwater	"	"	"	"	" "
3	"	During attack of blackwater ...	"	"	"	"	" "
4	"	At end of attack of blackwater ...	"	"	"	"	" "
5	"	Six days later ...	"	"	"	"	" "
6	3	Twenty hours before attack of haemoglobinuria	"	"	"	"	No autolysin present in blood plasma
7	"	During attack of haemoglobinuria ...	"	"	"	"	" "
8	"	During attack of haemoglobinuria ...	"	"	"	"	" "
9	"	At end of attack of haemoglobinuria...	"	"	"	"	" "
10	4	Three days after attack of haemoglobinuria	"	"	"	"	" "
11	"	Two days after relapse of haemoglobinuria	"	"	"	"	" "
12	5	Twelve hours after disappearance of haemoglobinuria	"	"	"	"	" "
13	6	At end of attack of haemoglobinuria	"	"	"	"	No isolysin present in blood plasma
14	6a	During attack of blackwater ...	"	"	"	"	No autolysin present in blood plasma
15	7	During attack of blackwater ...	"	"	"	"	" "
16	7a	During attack of haemoglobinuria ...	"	"	"	"	" "
17	8	During attack of haemoglobinuria ...	"	"	"	"	" "
18	9	Four days after attack of haemoglobinuria	"	"	"	"	Complete haemolysis of red cells*
19	"	Two months later ...	"	"	"	"	No autolysin present in blood plasma
20	10	During attack of haemoglobinuria ...	"	"	"	"	" "
21	"	During relapse of haemoglobinuria ...	"	"	"	"	" "
22	11	During attack of haemoglobinuria ...	"	"	"	"	" "
23	"	At end of attack of haemoglobinuria (during suppression)	"	"	"	"	" "
24	"	Three days later (during suppression)	"	"	"	"	" "
25	12	At end of attack of haemoglobinuria	"	"	"	"	Haemolysis of red cells†
26	"	One day later ...	"	"	"	"	No autolysin present in blood plasma
27	14	At end of attack of haemoglobinuria	"	"	"	"	" "
28	15	During attack of haemoglobinuria ...	"	"	"	"	" "
29	"	During attack of haemoglobinuria (during partial suppression)	"	"	"	"	" "
30	16	At end of attack of haemoglobinuria	"	"	"	"	" "

\* No turbidity, smell or other indication of putrefaction. Blood plasma examined ten hours after collection. Room temperature 28.5° C.

† Bacteria present in blood plasma, which was examined twenty-four hours after collection.

In Table 29 the result of testing, by the method above described, the plasma in sixteen attacks of blackwater fever, before, during and shortly after the paroxysm, is given, the examination being usually made within an hour of the withdrawal of blood. In addition, twenty-eight other observations upon healthy individuals, and also, for the sake of comparison, upon ten cases of malaria, are recorded in Table 30.

TABLE 30. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from malarial and other affections. Normal plasma is also employed in eleven observations.

No. of Observation	Condition of subject	Ratio of oxalated plasma to 2.5 % emulsion of red cells	Mixture kept at	For	Then kept at 37°C. for	Result
1	Healthy ... ..	20 : 1	0°C.-4°C.	30 min.	3 hrs.	No autolysin present in blood plasma
2	" 4 days later ...	"	"	"	"	" "
3	" 10 days later ...	"	"	"	"	" "
4	Healthy ... ..	"	"	"	"	" "
5	" 2 days later ...	"	"	"	"	" "
6	" 5 days later ...	"	"	"	"	No isolysin present in blood plasma
7	Healthy ... ..	"	"	"	"	" "
8	Healthy ... ..	"	"	"	"	" "
9	Healthy (Native) ...	"	"	"	"	" "
10	Healthy ... ..	"	"	"	"	" "
11	Healthy ... ..	"	"	"	"	" "
12	Typhoid ... ..	"	"	"	"	No autolysin present in blood plasma
13	Pyrexia (Native) ...	"	"	"	"	No isolysin present in blood plasma
14	Pleurisy (Native) ...	"	"	"	"	" "
15	Bullous eruption on thighs and legs (Native) ...	"	"	"	"	" "
16	Pneumonia (Native) ...	"	"	"	"	" "
17	Malaise (Native) ...	"	"	"	"	" "
18	Malaria ... ..	"	"	"	"	No autolysin present in blood plasma
19	Uraemia, T. 106° F. ...	"	"	"	"	No isolysin present in blood plasma
20	Malaria ... ..	"	"	"	"	Slight haemolysis of red cells *
21	" next day ... ..	"	"	"	"	No isolysin present in blood plasma
22	Malaria, T. 104° F. ...	"	"	"	"	" "
23	Malaria (Indian) ...	"	"	"	"	" "
24	Bilious fever ... ..	"	"	"	"	" "
25	Malaria ... ..	"	"	"	"	" "
26	Malaria ... ..	"	"	"	"	" "
27	Malaria ... ..	"	"	"	"	" "
28	Malaria ... ..	"	"	"	"	" "

\* Partial clotting of blood occurred during collection.

It will be seen that in all cases, with one exception, a negative result was obtained. In the cases of blackwater fever this negative result occurred, not only when the patient's plasma obtained during haemoglobinuria (twelve observations: 3, 7, 8, 14, 15, 16, 17, 20, 21, 22, 28, 29) was used, but also when that obtained both before blackwater appeared (three observations: 1, 2, 6) and (with one exception) shortly after haemoglobinuria had disappeared (nine observations: 4, 9, 10, 12, 13, 23, 25, 27, 30), as also (with one exception) some time after complete recovery had taken place (six observations: 5, 11, 18, 19, 24, 26). From these experiments the important conclusion follows, that the mechanism of production of blackwater stands in an altogether different category from that of paroxysmal haemoglobinuria. The presence of haemolysin or of defect of antilysin in the plasma, which is present in the latter case, is absent in blackwater fever, where, therefore, search must be made for other factors.

Table 30 shows that, as might be expected from the behaviour of the plasma of the blackwater fever patients, who had, in all the cases we examined, been the subjects of malaria, usually having had frequently repeated attacks, a negative result was also obtained in all but one of the cases of malaria dealt with (nine observations, 18, 20, 21, 22, 23, 25, 26, 27, 28).

In three cases a positive result was obtained. The first occurred in a patient suffering from blackwater fever, the plasma being taken four days after the cessation of haemoglobinuria (18, Table 29). When the plasma mixed with the patient's red cells was placed, after cooling for half an hour, in the incubator, slight haemolysis was visible at the end of twenty minutes, and complete haemolysis had occurred at the end of an hour and a half. When an examination of the same patient's plasma was made two months later, when he was in good health, though still troubled with occasional malarial attacks, a negative result was obtained (19, Table 29). The positive result obtained in this case was quite exceptional, and does not affect the general conclusion obtained with the rest of the blackwater fever cases. The second case (25, Table 29) occurred in another patient at the close of haemoglobinuria. In this case the examination was made twenty-four hours after collection of blood, and bacteria were

present, so that no conclusion as to presence or absence of haemolysin in the patient's blood can be drawn. It may here be observed that the growth of bacteria in oxalated plasma is not necessarily attended with haemolysis of red cells contained in the plasma; in contaminated plasma haemolysis is sometimes observed, but is not unfrequently absent. The third case (25, Table 30) occurred with a malarial patient's blood. Haemolysis was slight, and next day, on repeating the observation, no haemolysis was obtained.

The blood plasma of two oxen suffering from Texas fever was examined, on the day before death, during the passage of redwater. No autolysin was present in either case.

The blood plasma of two dogs suffering from piroplasmosis, both passing urine free from haemoglobin, was also examined. In each case no autolysin was found.

#### SUMMARY

The haemoglobinuria of blackwater fever is not dependent upon haemolysinaemia.



### III. THE RELATION OF HAEMOGLOBINAEMIA TO HAEMOGLOBINURIA IN BLACKWATER FEVER.

In the preceding sections it has been shown that the haemoglobinuria of blackwater fever cannot be attributed to the direct action of quinine on the red cells contained in the blood, nor is it associated ordinarily with the possession of a haemolytic power by the patient's blood plasma. These two possible factors in the mechanism of production of haemoglobinuria having been excluded, the way is clear for the consideration of the relationship of haemoglobinuria to haemoglobinaemia. Upon this point there is no consensus of opinion among writers, nor have different observers obtained concordant results respecting the existence of haemoglobinaemia in blackwater fever. Berthier\* and Hymans van der Bergh† were unable to observe red tinted serum during blackwater, though on spectroscopical examination oxyhaemoglobin bands were obtained; A. Plehn‡ found dissolved haemoglobin and also bile pigment in the blood plasma during blackwater; Bignami§ found no dissolved haemoglobin in the blood plasma during blackwater; Murri,¶ who is of opinion that haemoglobin cannot pass through the kidneys until the renal epithelium has undergone changes, states that haemoglobinaemia may persist for hours before haemoglobinuria appears. Stephens|| states guardedly: 'Not infrequently the serum shows no trace of haemoglobin, although the haemoglobinuria may be actually increasing. Haemoglobinaemia does however occur.' No quantitative determination of the amount of haemoglobin in the blood plasma in blackwater fever appears to have been made by any observer, however, and the views held in this connection appear to have been based entirely upon inspection of the serum. The only

\*Quoted by Marchiafava and Bignami, *Malarial Haemoglobinuria*, Twentieth Century Practice of Medicine, London, 1900, Vol. 19, p. 483.

† *Bydrage tot de kennis der Zwartwaterkorts*, Nederl. Tydschrift voor Geneeskunde, 1904.

‡ *Ätiologie und Pathogenese des Schwarzwasserfiebers*, Virch. Arch., 1903, B. 174, S. 509.

§ Marchiafava and Bignami, loc. cit.

¶ Quoted by Marchiafava and Bignami, loc. cit.

|| *A System of Medicine*. Allbutt and Rolleston, Vol. II., Pt. 2, p. 297, London, 1907.

experimental work dealing with the relationship in question seems to be that of Ponfick,\* who found that in haemoglobinaemia, haemoglobinuria occurred only when the amount of haemoglobin set free in the blood exceeded one-sixtieth of the total amount contained in the red cells. If less were set free, then the activity of the liver was sufficient to convert the haemoglobin into the constituents of the bile, in particular into bile colouring matter, whereby hypercholia, icterus and dark coloration of the urine were produced.

The reinvestigation of this subject, which it was decided to undertake, includes two distinct enquiries: (1) Is haemoglobinaemia present in blackwater fever? (2) Is haemoglobinuria readily producible as the result of haemoglobinaemia, and, if so, is there any quantitative relationship between the two?

The first enquiry involved the examination of the blood plasma in blackwater fever before, during and shortly after the haemoglobinuria. As control observations the plasma of normal persons, who had never had blackwater fever, was examined, and in order to afford further comparison the plasma of individuals suffering from malaria and other morbid conditions was studied.

*Method.* The mode of examination was as follows:—The finger, having been previously carefully cleansed and dried, was pricked with a bayonet pointed needle and blood allowed to fall drop by drop into a collecting vessel containing 0.1 c.cm. of a 1 per cent. solution of potassium oxalate, about 0.5 c.cm. (ten drops) of blood being collected. Without any delay the oxalated blood was centrifugalised until the red cells were completely precipitated. The supernatant plasma was pipetted off and transferred to a small glass cylinder 18 mm. high and 4 mm. in internal diameter. This was then placed under a double spectroscope, a cell, the height of which could be varied, filled with a solution of haemoglobin of suitable concentration, being placed in the path of the second spectrum, and the height of the column of the solution it contained altered until the oxyhaemoglobin bands which it produced matched in point of intensity those given by the plasma. The concentration of the haemoglobin solution was determined by means of a haemoglobino-meter reading, the absolute values of the scale of the instrument

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\*Virch. Arch., 1875, B. 62, S. 273; Berl. kl. Wochenschr., 1883, No. 26.

having been determined in terms of the red cells (in the moist condition) of one of us taken as a standard.

When healthy human blood was collected in the manner above described, the plasma was found to be light orange coloured and to present no trace of a red tint in a tube of about 5 mm. internal diameter. If examined by the spectroscope in a column 18 mm. high, oxyhaemoglobin bands were always found, not unfrequently, it is true, on the threshold of visibility. When matched with a solution of haemoglobin, the amount present was represented by percentages of

TABLE 31. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in healthy men.

No. of Observation	Condition of subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectroscope	Percentage of haemoglobin in the blood plasma
1	Healthy ... ..	4 : 1	Orange	18 mm.	0.25 %
2	" (twelve days later)	"	Orange	"	0.13 %
3	" (nine months later)	"	Light orange	"	0.15 %
4	Healthy ... ..	"	Light orange	"	0.10 %
5	" (one day later)	"	Light orange	"	0.13 %
6	" (seven days later)	"	Light orange	"	0.13 %
7	Healthy ... ..	"	Light orange	"	0.10 %

red cells ranging from 0.25 per cent. to 0.10 per cent. or less (Table 31). When the serum is light orange in colour the limit of visibility of the oxyhaemoglobin bands, it may be observed, is in a column 18 mm. high about 0.02 per cent. in daylight of moderate intensity. The haemoglobin present was not due to the red blood cells having been incompletely removed by centrifugalisation, for microscopical examination, aided by further centrifugalisation, failed to reveal the presence of red cells, while in concentrations of 0.1 per cent. or more, due to the presence of red cells, the plasma was obviously turbid to the naked eye, and when examined with a hand lens red cells were readily seen in fair abundance. On the other hand, it is clear that the amount of haemoglobin present is dependent to some extent upon the technique, for if two samples of blood are withdrawn from the finger in succession, the intensity of the bands in the plasma will be found as a rule to exhibit some variation, which is, however,

relatively slight in amount. The cause of such variation is not easy to determine. The amount of squeezing to which the finger may be subjected stands in no definite relation to the intensity of the bands present, though mechanical injury to the red cells, as in whipping blood, produces some degree of laking; nor does the length of time (up to one and a half hours) during which the oxalated blood is kept before centrifugalisation ordinarily affect the strength of the bands in any definite manner. The only circumstance which

TABLE 32. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in patients not suffering from blackwater fever.

No. of Observation	Condition present in subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectroscope	Percentage of haemoglobin in the blood plasma
1	Malaria (Indian) ... ..	4 : 1	Light orange	18 mm.	0.31 %
2	T. 101° F. ... ..	"	Light orange	"	0.13 %
3	Gummata of liver ... ..	"	Light orange	"	0.13 %
4	Herpes Zoster ... ..	"	Light orange	"	0.10 %
5	Malaria ... ..	"	Light orange	"	0.13 %
6	Malaria ... ..	"	Light orange	"	0.10 %
7	" (six days later) ... ..	"	Light orange	"	0.10 %
8	" (seven days later) ... ..	"	Light orange	"	0.31 %
9	Malaria (Indian) ... ..	"	Light orange	"	0.06 %
10	Malaria ... ..	"	Light orange	"	0.13 %
11	Typhoid ... ..	"	Light orange	"	0.16 %
12	Malaria ... ..	"	Light orange	"	0.02 %
13	Malaria ... ..	"	Dark orange	"	0.09 %
14	" (one day later) ... ..	"	Dark orange	"	0.20 %
15	Diarrhoea ... ..	"	Light orange	"	0.05 %
16	T. 102° F. ... ..	"	Light orange	"	0.04 %
17	Dysentery ... ..	"	Orange	"	0.16 %
18	Dysentery ... ..	"	Light orange	"	0.04 %
19	Malaria ... ..	"	Light orange	"	0.15 %
20	Malaria ... ..	"	Orange	"	0.05 %
21	Malaria ... ..	"	Light orange	"	0.07 %

constantly affected the haemoglobin bands was the occurrence of clotting. If a clot formed, the serum expressed always showed stronger haemoglobin bands than the plasma, and if a sterile clot was allowed to stand for three hours the serum in contact with it usually showed in a thickness of 5 mm. to 7 mm., a reddish tint just recognisable with the naked eye. The haemoglobin bands, moreover, are deepest in those portions of the serum, which are last exuded. If the clot is broken up with a glass rod the red coloration becomes deeper.



TABLE 33. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in blackwater fever patients.

No. of Observation	Condition of subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectro-scope	Percentage of haemoglobin in blood plasma
1	Case 2—Sixteen hours before attack of blackwater	4 : 1	Dark orange	18 mm.	0.13 %
2	" Two hours before attack of blackwater	"	Dark orange	"	0.13 %
3	" During attack of blackwater ...	"	Dark orange	"	0.13 %
4	" At end of attack of blackwater...	"	Dark orange	"	0.13 %
5	Case 3—Twenty hours before attack of haemoglobinuria	"	Dark orange	"	0.16 %
6	" During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.57 %
7	" During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.40 %
8	" At end of attack of haemoglobinuria ...	"	Orange	"	0.08 %
9	" Twenty-four hours later ...	"	Orange	"	0.08 %
10	" Seven days later ...	"	Light orange	"	0.11 %
11	Case 4—At end of attack of haemoglobinuria ...	"	Dark orange	"	0.06 %
12	" Two days after a relapse of haemoglobinuria	"	Light orange	"	0.06 %
13	Case 5—At end of attack of haemoglobinuria ...	"	Light orange	"	0.13 %
14	Case 6—At end of attack of haemoglobinuria ...	"	Dark orange	"	0.13 %
15	Case 6a—At end of attack of blackwater ...	"	Light orange	"	0.16 %
16	Case 7—During attack of blackwater ...	"	Dark orange	"	0.13 %
17	" During convalescence, three days later	"	Light orange	"	0.06 %
18	Case 7a—During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.74 %
19	Case 8—During attack of haemoglobinuria ...	"	Dark orange	"	0.13 %
20	" At end of attack of haemoglobinuria ...	"	Dark orange	"	0.07 %
21	Case 10—During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.85 %
22	" Towards close of attack of haemoglobinuria	"	Dark orange	"	0.09 %
23	" During slight relapse of haemoglobinuria	"	Dark orange	"	0.30 %
24	" During another slight relapse of haemoglobinuria	"	Dark orange	"	0.20 %
25	Case 11—Towards close of attack of haemoglobinuria	"	Dark orange	"	0.25 %
26	" At end of attack of haemoglobinuria (during suppression)	"	Dark orange	"	0.16 %
27	" Three days later (during suppression)...	"	Light orange	"	0.10 %
28	Case 12—At end of attack of haemoglobinuria ...	"	Dark orange	"	0.20 %
29	" Next day ...	"	Orange	"	0.14 %
30	Case 14—During attack of haemoglobinuria ...	"	Light red	"	0.57 %
31	" Ten hours after close of attack ...	"	Orange	"	0.18 %
32	Case 14a—Towards close of attack of haemoglobinuria	"	Dark orange	"	0.20 %
33	Case 15—During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.56 %
34	" During attack of haemoglobinuria ...	"	Dark orange	"	0.42 %
35	Case 16—At end of attack of haemoglobinuria ...	"	Dark orange	"	0.20 %
36	Case 17—During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.65 %
37	" During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.67 %
38	" During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.95 %
39	" During attack of haemoglobinuria ...	"	Dark orange with reddish tint	"	0.48 %
40	" At end of attack of haemoglobinuria ...	"	Dark orange	"	0.18 %

When the blood of individuals, not in normal health, but suffering from malaria or other affections met with in the tropics, was examined it was found that the variations in the amount of haemoglobin dissolved in the blood plasma lay within much the same limits as in health (Table 32). Thus in fourteen cases of malaria the percentage of dissolved haemoglobin in the blood plasma ranged in twelve cases between 0.02 per cent. and 0.20 per cent., but reached in two cases the somewhat high degree of 0.31 per cent. (in neither of the two latter did blackwater fever make its appearance); in the remaining seven cases (dysentery, diarrhoea, etc.) the range was between 0.04 per cent. and 0.16 per cent. It may here be noted that in perfectly healthy rabbits and oxen we have failed to find dissolved haemoglobin in the oxalated blood plasma. Examined in the above described manner it was found that the blood plasma in blackwater fever gave percentages of dissolved haemoglobin ranging from less than 0.06 per cent. to 0.95 per cent. (Table 33). The number of cases examined was seventeen, and the number of estimations made amounted to forty. The observations fall into three classes: those made before, after and during the passage of blackwater. In the first class, which includes only three observations (1, 2, 5), the percentage of dissolved haemoglobin was 0.13 per cent. or less. In the second class, which includes eighteen observations (4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 26, 27, 28, 29, 31, 35, 40), usually made shortly after the close of the attack, the percentage of dissolved haemoglobin was from 0.20 per cent. to 0.06 per cent. In the third class, consisting of nineteen observations given in Table 33 in thick type, the percentage of dissolved haemoglobin was in twelve (6, 7, 18, 21, 23, 30, 33, 34, 36, 37, 38, 39) cases raised lying between 0.95 per cent. and 0.30 per cent.; in the remaining seven (3, 16, 19, 22, 24, 25, 32) observations it lay within the limits met with in health, namely, 0.25 per cent. to 0.09 per cent. With regard to the latter, it must be remarked that although in these observations the first specimen of urine voided after the examination of the blood plasma contained haemoglobin, as will be seen by reference to the clinical histories on pp. 176 to 246; nevertheless it does not follow that the urine which was being excreted at the time of examination of the blood plasma then contained haemoglobin. It is possible, if not probable, that in some cases the haemoglobin had already disappeared, though, owing to the relatively long intervals

at which the patient passed urine, this did not become evident. Much variation, it may be observed, was exhibited in respect of the passage of urine, in some attacks urine being passed frequently, in others retained long in the bladder. Failure to observe haemoglobinaemia—assuming for the moment that this is constantly present during haemoglobinuria, a point which will be further considered on pp. 86 to 89—would, therefore, be expected to occur in a certain proportion of the cases examined during the passage of urine containing haemoglobin.

TABLE 34 Examination of blood plasma for the presence of dissolved haemoglobin, during haemoglobinuria of blackwater fever. Selected from Table 33.

No. of observation	Case	Percentage of haemoglobin in plasma	Condition of urine at time of collecting plasma.			
3	2	0.13 %	On centrifugalising the urine the supernatant liquid was found to be brown in colour, gave no oxyhaemoglobin bands, but yielded $\frac{1}{20}$ col. of brown precipitate on boiling after acidification with acetic acid (= about 0.2 % of haemoglobin).			
16	7	0.13 %	Similar to above			
19	8	0.13 %	Urine contained 0.1 % of dissolved haemoglobin. See Fig. 12			
22	10	0.09 %	"	0.03 %	"	13
25	11	0.25 %	"	0.9 %	"	14
32	14a	0.20 %	"	0.24 %	"	17
6	3	0.57 %	"	1.2 %	"	10
7	"	0.40 %	"	1.3 %	"	"
18	7a	0.74 %	"	0.4 % to 1.1 %	"	11
21	10	0.85 %	"	1.1 %	"	13
23	"	0.30 %	"	1.5 %	"	"
24	"	0.20 %	"	0.4 %	"	"
30	14	0.57 %	"	1.5 %	"	16
33	15	0.56 %	"	1.3 %	"	18
34	"	0.42 %	"	0.7 %	"	"
36	17	0.65 %	"	3.1 %	"	20
37	"	0.67 %	"	1.4 %	"	"
38	"	0.95 %	"	1.9 %	"	"
39	"	0.48 %	"	0.5 %	"	"

Further support to this view is afforded by Table 34, in which the percentages of dissolved haemoglobin in the blood plasma in the nineteen observations last referred to are given side by side with the corresponding percentages of haemoglobin in the urine. It will be seen that, in the cases (3, 16, 19, 22, 24, 25, 32) in which the percentage



of haemoglobin in the blood plasma is not increased, the percentage of haemoglobin in the urine is low, reaching to 0.9 per cent. in one case (25) and lying in the remaining six cases between 0.4 per cent. and 0.03 per cent. On the other hand, in the twelve observations in which the amount of dissolved haemoglobin in the blood plasma was increased, the percentage of haemoglobin in the urine was much higher, ranging between 0.4 per cent. and 3.1 per cent.

To sum up, then, we may say that, in the observations made in blackwater fever while the urine in the bladder still contained haemoglobin, an increased amount of dissolved haemoglobin in the blood plasma was observed in most but not in all cases.

We now turn to the second problem: is haemoglobinaemia ordinarily attended with haemoglobinuria, and, if so, what quantitative relationship between the two conditions exists? To determine this point it was necessary to produce haemoglobinaemia experimentally.

*Method.* Oxalated rabbit's blood was centrifugalised, the supernatant plasma pipetted off, and the red cells laked by adding a small amount of distilled water. To the solution thus obtained an amount of solid sodium chloride was added in amount sufficient to make the proportion of this salt 0.85 per cent. The red cell stromata, which were precipitated by the addition of sodium chloride, were then removed by centrifugalisation, a dark red liquid being obtained. The percentage of haemoglobin in this solution was determined by means of a haemoglobinometer reading, and a measured volume of the solution injected into a vein of the ear of the rabbit supplying the haemoglobin. At the end of three to six or more minutes, about ten drops of blood from the opposite ear were allowed to fall into 0.1 c.cm. of 1 per cent. potassium oxalate solution, the volume of the mixture being carefully measured. This was then centrifugalised, and the percentage of haemoglobin in the plasma determined usually by matching, under a comparison spectroscope, with a solution of haemoglobin of known concentration, though when the concentration of haemoglobin was high a haemoglobinometer determination was sometimes made instead. At later periods similar estimations of the haemoglobin content of the plasma were carried out. In the same way the haemoglobin content of the urine was determined, and the



urine was also tested for coagulable proteid, any deposit present being submitted to microscopical examination. In Experiments 8, 9 and 10 the urine was collected continuously by means of a cannula introduced into the bladder, in the other experiments it was voided naturally.

Before experiment, the oxalated blood plasma of the rabbits employed was found to be of a very faint yellow tint, almost colourless. On spectroscopic examination no haemoglobin bands were as a rule seen, though on one occasion 0.05 per cent. of dissolved haemoglobin was met with. The weight of the rabbits ranged from 912 g. to 1,760 g., the total amount of blood the animals possessed varied from 75 c.cm. to 118 c.cm., and the ratio of blood to body weight (Table 35) was  $\frac{1}{10}$  to  $\frac{1}{20}$ , the higher fraction being reached in the younger, and therefore lighter, animals.

The amount of haemoglobin injected was that obtainable from 0.44 g. to 7.52 g. of red cells (in the moist condition). In all except Experiments 6 and 8, Tables 35 and 37, the haemoglobin was obtained from the animal's own red cells. The amount required in the two latter experiments being too large to be conveniently taken from the animals subjected to experiment, part of the haemoglobin injected was obtained from other rabbits. The injection of laked blood produced no recognisable ill effect, the animals taking food well during the experiment and seeming in every respect normal. In Experiment 5 the animal was apparently unaffected by the injection, but died suddenly at the end of eighty-five minutes; possibly in this case death was due to embolism, for the stromata of the laked red cells were not completely removed from the haemoglobin solution before injection.

After the injection of dissolved haemoglobin into the vein of the ear, the blood plasma of the injected animal became of a reddish colour. At the end of three to twenty minutes, estimations of the amount of haemoglobin present showed that this ranged from 0.78 per cent. to 10.80 per cent. The amount present immediately after injection may be calculated in the manner indicated on p. 90, and is found to be somewhat higher, ranging between 0.81 per cent. and 12.15 per cent. As the amount of haemoglobin injected intravenously was known, the total volume of the plasma of the rabbit could be

TABLE 35. Experimental haemoglobinuria in rabbits.

No. of experiment	Amount of haemoglobin injected intravenously expressed in terms of healthy human wet red cells	Percentage of haemoglobin dissolved in blood plasma		Percentage of haemoglobin dissolved in urine		Total haemoglobin in urine	Remarks
1	1.4 g.	0.5% at end of 60 min.	0.6% " 540 "	Trace in urine passed at end of 420 mins.			General condition unaffected during experiment
2	0.81 g.	0.81% (calculated) immediately after injection	0.78% at end of 10 mins. after injection	0.09%	" 250 "	0.023 g.	" "
		0.49% " 120 "	0.26% " 520 "	Absent	" 1320 "		
		0.06% " 1340 "					
3	3.15 g.	5.04% (calculated) immediately after injection	4.59% at end of 11 mins. after injection.	1.70%	" 52 "	0.524 g.	" "
		3.46% " 36 "	2.12% " 98 "	0.62%	" 1233 "		
		1.81% " 166 "	1.12% " 218 "	0.35%	" 1506 "		
		0.873% " 279 "	0.237% " 476 "	Absent	" 1900 "		
		0.145% " 1346 "					
4	5.39 g.	6.50% (calculated) immediately after injection	5.03% at end of 27 mins. after injection	1.32%	" 135 "	1.108 g.	" "
		4.50% " 73 "	1.76% " 134 "	0.93%	" 562 "		
		1.60% " 206 "	1.65% " 257 "	Absent	" 1582 "		
		1.05% " 334 "	0.64% " 488 "				
		0.14% " 1386 "					
5	3.12 g.	4.34% (calculated) immediately after injection	4.17% at end of 21 mins. after injection	2.45%	" 85 "	0.12 g.	Died suddenly at the end of 85 mins.
		4.01% " 45 "	3.17% " 85 "				
6	7.52 g.	8.59% (calculated) immediately after injection	8.10% at end of 8 mins. after injection	0.633%	" 744 "	0.80 g.	General condition unaffected during experiment
		5.05% " 62 "	3.26% " 117 "	Absent	" 1556 "		
		2.63% " 180 "	1.68% " 237 "				
		1.30% " 297 "	0.84% " 358 "				

No. of experiment	Amount of haemoglobin injected intravenously expressed in terms of healthy human wet red cells	Percentage of haemoglobin dissolved in blood plasma	Percentage of haemoglobin dissolved in urine	Total haemoglobin in urine	Remarks
7	2.52 g.	5.13% (calculated) immediately after injection 4.92% at end of 6 mins. after injection 1.85% " " 151 "	4.80% in urine passed at end of 125 minutes	0.29 g.	General condition unaffected during experiment
	7.00 g. injected 19½ hours later	9.20% (calculated) immediately after injection 7.90% at end of 33 mins. after 2nd injection 3.54% " " 206 " 0.78% " " 544 "	2.50% " " 276 "	1.00 g.	" "
	5.70 g. injected after a further period of 8½ hours. Total in 28 hours, 15.22 g.	8.50% at end of 67 mins. after 3rd injection	4.50% " " 79 " 5.00% " " 800 " Absent " " 1432 "	1.13 g. 1.5 g.	" "
	7.33 g.	12.15% (calculated) immediately after injection 10.80% at end of 17 mins. after injection 6.72% " " 62 " 5.70% " " 88 " 5.30% " " 118 " 4.65% " " 152 " 3.52% " " 172 " 2.56% " " 315 " 2.00% " " 383 " 1.60% " " 442 " 1.30% " " 498 "	7.20% " " 28 " 6.78% " " 40 " 7.16% " " 60 " 6.54% " " 86 " 8.22% " " 150 " 11.16% " " 171 " 8.88% " " 260 " 7.60% " " 380 " 5.52% " " 436 " 4.70% " " 501 "	1.34 g.	" "
9	1.3 g.	2.13% (calculated) immediately after injection 2.10% at end of 13 mins. after injection  1.50% " " 168 "	1.51% " " 6 " 5.30% " " 17 " 5.25% " " 43 " 11.20% " " 63 " 9.80% " " 98 " 6.24% " " 183 " 1.10% " " 343 " Trace " " 478 "	0.33 g.	" "
	0.44 g.	0.96% (calculated) immediately after injection 0.90% at end of 5 mins. after injection  0.54% " " 41 " 0.31% " " 79 " 0.26% " " 132 "	Nil " " 17 " 2.14% " " 27 " 6.96% " " 37 " 2.90% " " 52 " 1.59% " " 67 " 0.43% " " 82 " Nil " " 92 " Nil " " 107 "	0.08 g.	" "

calculated from the percentage present in the plasma after injection, and, as the relation between the volume of the red cells and that of the plasma had previously been determined by the aid of the haemocrit, the total volume of the blood was also by this means ascertained.\* As time went on the amount of haemoglobin in the rabbit's plasma diminished at first rapidly, then more slowly, until at the end of twenty-four hours the amount present was less than 0.2 per cent.

The first specimen of the urine, after intravenous injection of dissolved haemoglobin, was, in all experiments except the first two, in which the tint was lighter, porter coloured in a layer two inches or more in thickness, and dark red or reddish brown in a layer one centimetre thick. The percentage of haemoglobin ranged from 5.0 per cent. to 0.6 per cent., reaching, however, in one experiment 0.09 per cent. In succeeding specimens of the urine the amount of haemoglobin usually became progressively smaller, and at the end of twenty-four hours disappeared. The total amount of haemoglobin eliminated in the urine after injection, ranged in the experiments made between 0.2 g. and 1.34 g. These amounts refer, however, only to unaltered haemoglobin. In addition a relatively small amount of haemoglobin was broken up in the urine, to which it imparted a brownish tint. The actual amount of haemoglobin passing into the urine would, therefore, be slightly greater than the amounts given in Column 6, Table 35. To obtain some idea of the total haemoglobin discharged into the urine, a given volume of the urine was rendered slightly acid with acetic acid, boiled, and the volume of the resulting chocolate coloured precipitate measured after centrifugalisation in a graduated tube. By comparison with a parallel series of experiments in which known amounts of red cells were laked and added to urine, the volume of the precipitate obtained after acidifying and boiling being carefully noted, the amounts of haemoglobin originally discharged into the urine were determined. The percentages obtained in this way were larger than those given by haemoglobino-meter readings of the diluted urine, by an amount not exceeding 25 per cent. In these experiments the precipitate produced in the

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\* For further details cp. A method of estimating the total volume of blood contained in the living body, *Proceedings of the Royal Society of London*, 1909, Series B., vol. 81.



urine by boiling was indistinguishable from that obtained on boiling a solution of haemoglobin. In particular, no lighter colour, such as might be taken to indicate the presence of serum albumin or globulin could be detected, when the former precipitate was compared with the latter.

TABLE 36. Examination of oxalated blood plasma for the presence of dissolved haemoglobin, in experimentally induced haemoglobinuria in rabbits. Selected from Table 35.

No. of Experiment	Percentage of haemoglobin in plasma	Condition of urine
1	0.5 % or less	Urine contained trace of haemoglobin
2	0.81 % to less than 0.49 %	" " 0.09 % " See fig. 2
3	5.04 % to 3.10 %	" " 1.70 % " " 3
4	6.50 % to 1.76 %	" " 1.32 % " " 4
5	4.34 % to 3.17 %	" " 2.45 % " " 5
6	8.59 % to less than 0.84 %	" " 0.63 % " " 6
7	(a) 5.13 % to 2.20 %	" " 4.80 %
	(b) 9.20 % to less than 3.54 %	" " 2.50 %
	(c) 8.50 % or more	" " 4.50 %
8	12.15 % to 10.4 %	" " 7.20 % " 7
9	2.20 % to 2.00 %	" " 11.20 % (maximum) " 8
10	0.90 % to 0.54 %	" " 7.00 % (maximum) " 9

In Table 36 some of the percentages of haemoglobin in blood plasma and urine are placed side by side so that the degree of haemoglobinaemia and haemoglobinuria may be compared, as was done in the case of the blackwater fever observations recorded in Table 34. The highest degree of haemoglobinaemia observed in the rabbit is 10.8 per cent., while the highest amount in blackwater fever was 0.95 per cent. It must, however, be observed that in the former case the observations were frequently repeated, and the time at which the maximum would occur was known in advance, while in the human subject the number of observations which could be made was limited and the probable course of the haemoglobinaemia was unknown, so that highest percentages were therefore in all probability not ascertained.

In comparing the degree of haemoglobinaemia with that of haemoglobinuria in experimentally induced haemoglobinaemia it must be remembered that the last three experiments in Table 36 stand apart from the others since in these the urine was collected

continuously, instead of being voided at intervals as in the preceding experiments. It will be noticed that the percentage of haemoglobin in the urine voided in the first seven experiments is less than that in the blood plasma (Table 36); in Observation 8 the same is also noted, but in Observations 9 and 10 this relation is reversed in the sense that the maximum percentage of haemoglobin in the urine is considerably higher than the corresponding percentage in the blood plasma. A better comparison is, however, made if curves are constructed, the ordinates of which denote percentages of haemoglobin and the abscissae periods of time. This is done in Figs. 2 to 9.

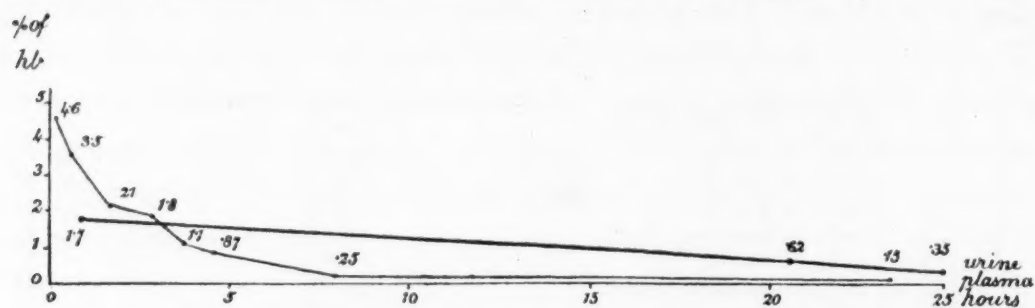


FIG. 2. Exp. 3, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

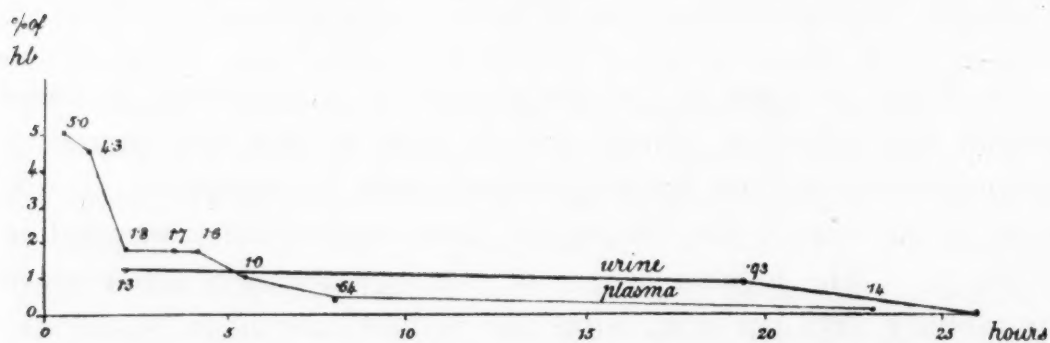


FIG. 3. Exp. 4, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

In most of these curves (Figs. 2 to 6) the percentage of haemoglobin in the plasma appears first greater, and subsequently less, than that in the urine. In Figs. 7, 8 and 9, however, it is seen that the percentage of haemoglobin in the urine in reality at the beginning of the experiment quickly rises till it considerably exceeds that in the plasma, after which it declines again with the latter. The greatest difference was observed in Experiment 9 in which the percentage of haemoglobin in the urine became at one time as much as 7 per cent., while that in the blood plasma was only 0.54 per cent.

It is, of course, obvious that if in these three experiments (Figs. 7, 8, 9) the urine had been allowed to accumulate in the bladder sufficiently long no such relation would have been discoverable. As

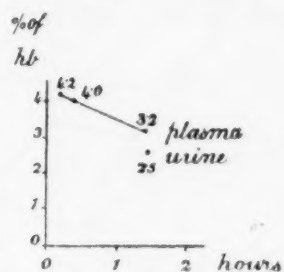


FIG. 4. Exp. 5, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

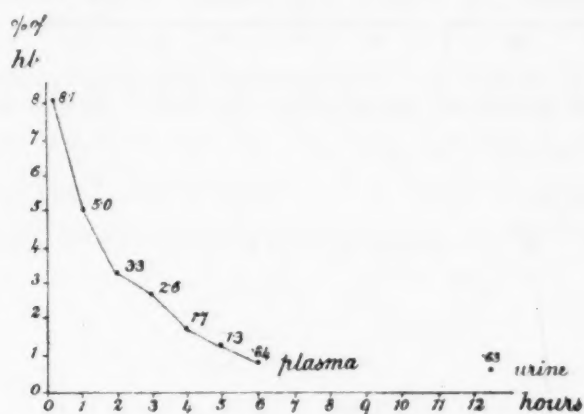


FIG. 5. Exp. 6, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

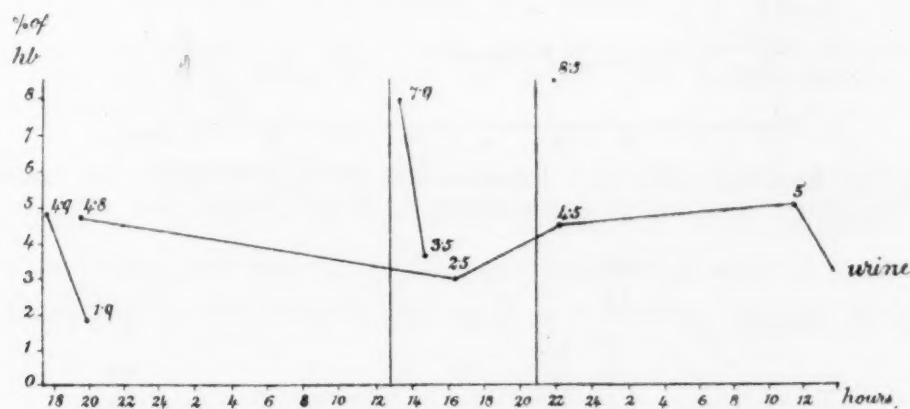


FIG. 6. Exp. 7, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

regards the earlier portions of the urine, which contained less haemoglobin than the blood plasma, in Experiments 8 to 10, in which continuous collection was practised, it is uncertain how far these represent the real state of affairs, since although the urine was

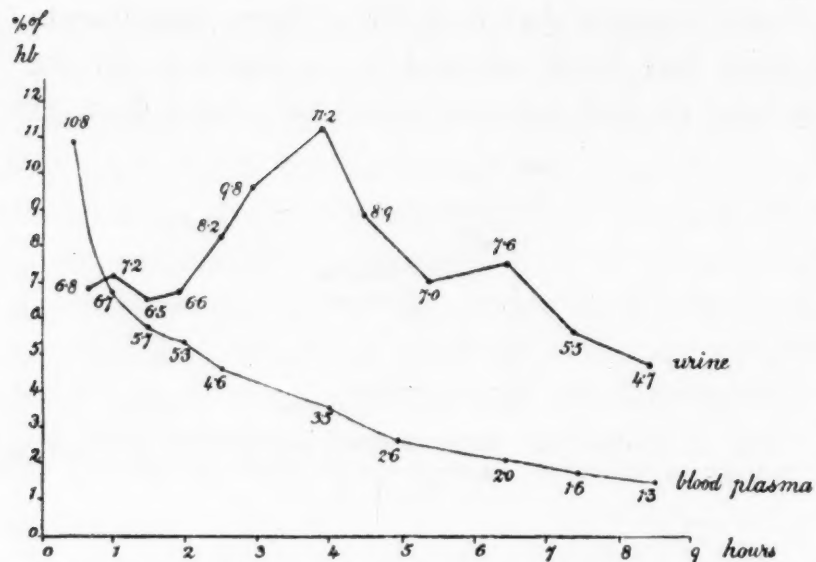


FIG. 7. Exp. 8, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

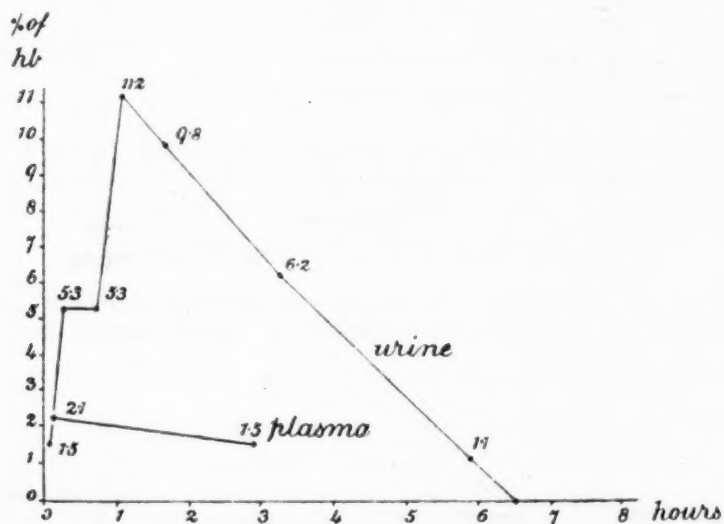


FIG. 8. Exp. 9, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

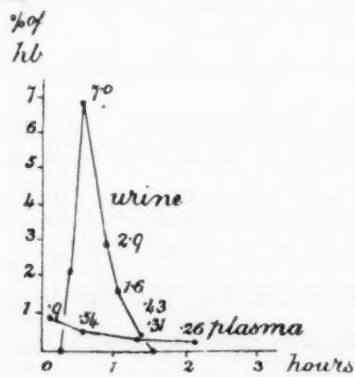


FIG. 9. Exp. 10, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.



collected by means of a cannula tied in the bladder, the portion first secreted containing haemoglobin would be diluted with the normal urine still remaining in the pelvis of the kidneys and ureters, and also with the very small amount yet remaining in the bladder. In

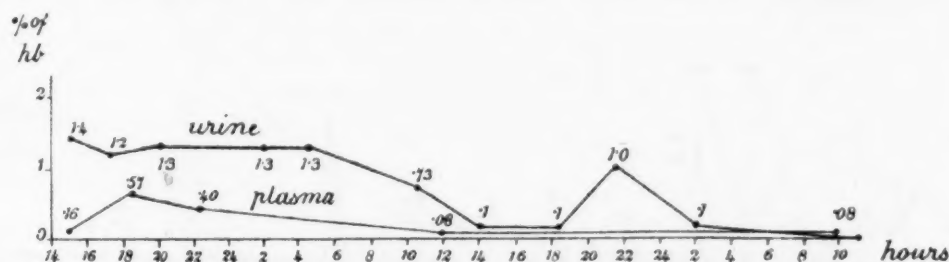


FIG. 10. Blackwater Fever, Case 3. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 185.

the experiments recorded in Figs. 7 to 9, the urine in the glass cannula continued amber-coloured for two to five minutes after intravenous injection of dissolved haemoglobin; it then became dark red in colour, a sharp line of demarcation appearing in the cannula between

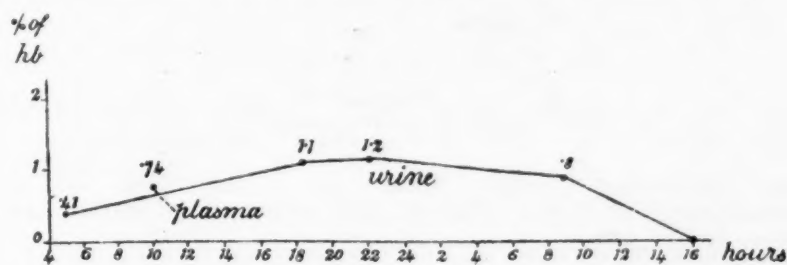


FIG. 11. Blackwater Fever, Case 7a. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 199.

the two kinds of urine. The red tint of the urine then underwent a slight increase in depth as the urine continued to be secreted. To what extent this was due to the first portions being diluted with urine still remaining in the urinary passages is not clear, though from the

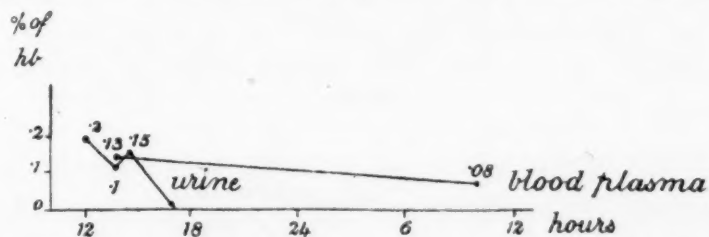


FIG. 12. Blackwater Fever, Case 8. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 204.

curves in Figs. 7 to 9 it can be asserted with certainty that the initial rate of elimination of urine is not the maximum rate, but continues for a time to increase.

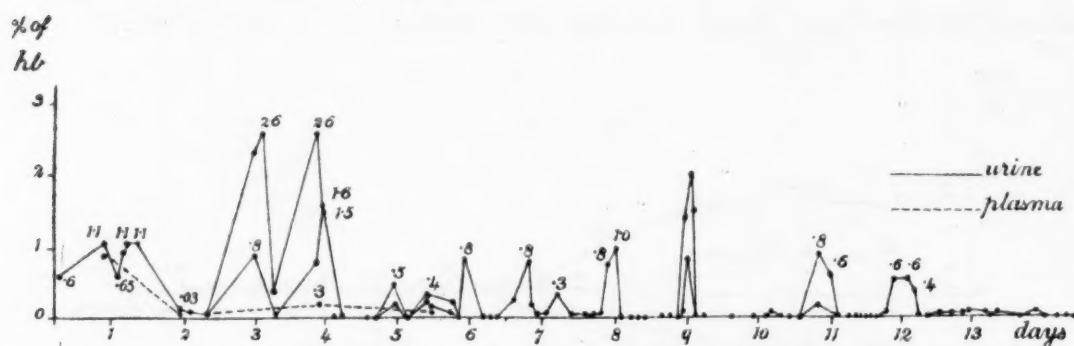


FIG. 13. Blackwater Fever, Case 10. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 211.

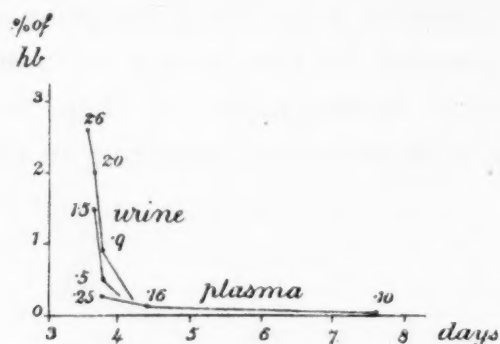


FIG. 14. Blackwater Fever, Case 11. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 220.

It is now possible in the light of the preceding experimental observations to study further the relation between the haemoglobinuria and the accompanying haemoglobinaemia already observed in some, but not all, of the cases of blackwater fever

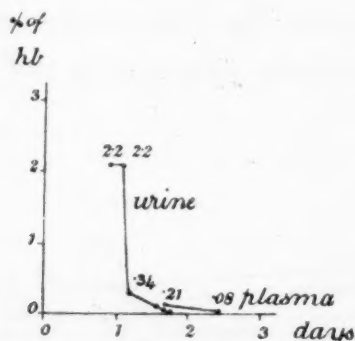


FIG. 15. Blackwater Fever, Case 12. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 224.

examined during the passage of urine containing haemoglobin. Turning again to Table 34 (p. 75), we find that we are here dealing with much smaller percentages of haemoglobin, especially in the case

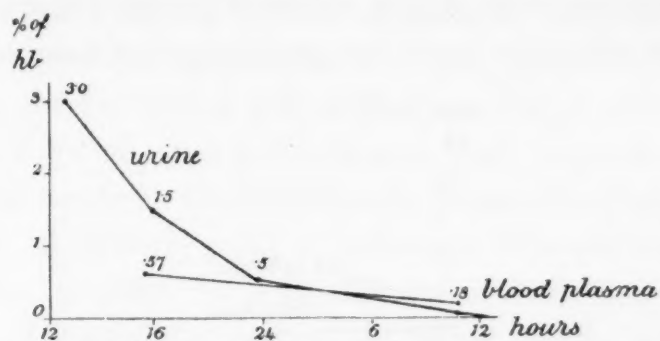


FIG. 16. Blackwater Fever, Case 14. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 228.

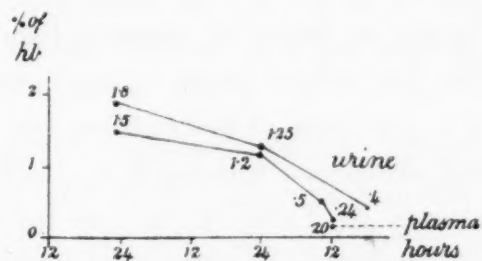


FIG. 17. Blackwater Fever, Case 14a. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 230.

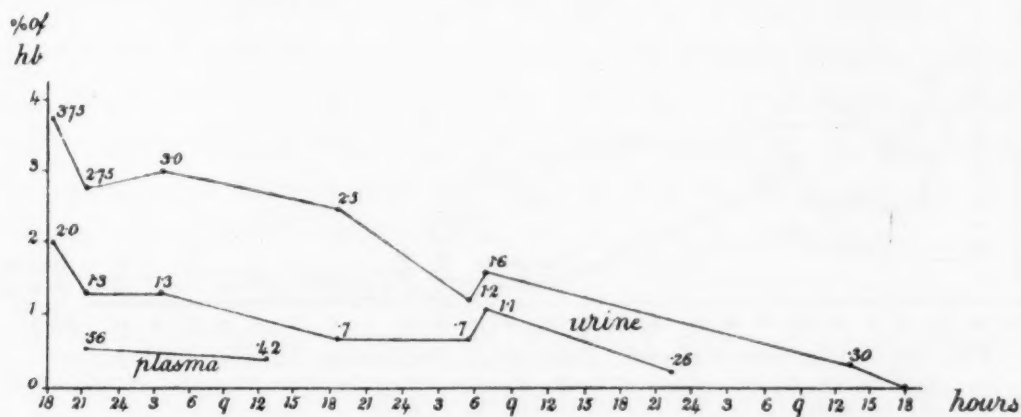


FIG. 18. Blackwater Fever, Case 15. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 233.

of the blood plasma, than those obtaining experimentally in rabbits. Although the observations made on blackwater fever are, in part, necessarily far less complete than those obtaining in the rabbit, and the percentages obtained cannot pretend to represent maximal

percentages, yet one feature stands out quite clearly, namely, that the percentage of dissolved haemoglobin in the blood plasma appears to be much lower than in experimentally induced haemoglobinaemia, none of the specimens of plasma obtained giving a higher percentage than 0.95. On the other hand the percentages of haemoglobin in the

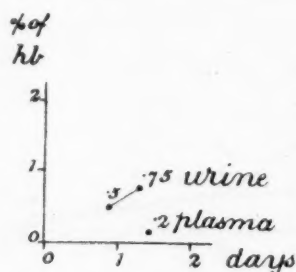


FIG. 19. Blackwater Fever, Case 16. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 237.

urine, though not quite so high as those observed under similar conditions in rabbits, are much more nearly comparable with the latter, the highest 3.1 per cent. (Observation 36, Table 34). That the degree of haemoglobinaemia observed in blackwater fever in the last thirteen experiments in Table 34 is not incompatible with the considerably higher percentages of haemoglobin met with in the urine

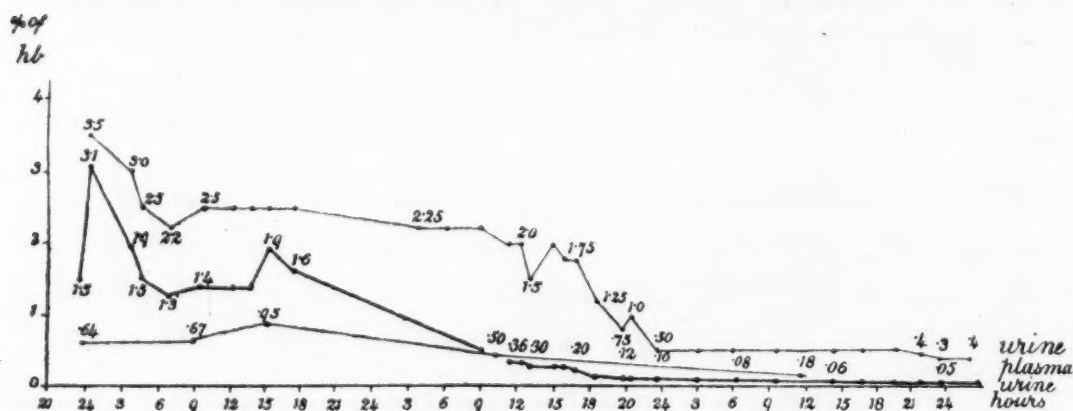


FIG. 20. Blackwater Fever, Case 17. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 241.

in these cases, is shown by the experimental work in rabbits, in particular by Experiments 7 to 9, Table 36, so that there is nothing in these thirteen results (Observations 6 to 39, Table 34) to negative the supposition that the haemoglobinuria in these cases is dependent upon accompanying haemoglobinaemia. To render a comparison with the experimental results easier the series of diagrams, Figs. 10



to 20, have been made. Notwithstanding the incompleteness of some of the Figures, they illustrate the wide divergence in degree between haemoglobinaemia and haemoglobinuria just referred to (Figs. 10, 13, 14, 16, 18, 20). It is observable that a fall in the percentage of dissolved haemoglobin in the blood plasma appears attended by a corresponding fall in the percentage of haemoglobin in the urine (Figs. 10, 12, 13, 16, 18, 20), just as occurs, after the initial maximum has been reached, in experimental haemoglobinaemia (Figs. 8, 9, 10). A striking point of difference between the two series of observations is that in experimental haemoglobinaemia the disappearance of haemoglobin from the blood plasma appears to be more rapid than in blackwater fever. To make this clear, a comparison in respect of time should be made between the rate of disappearance of haemoglobin in Figs. 2 to 9 (experimental haemoglobinaemia) and that occurring in Figs. 10, 12, 13, 14, 18 and 20 (blackwater fever).

If the comparison is made between the amount of haemoglobin injected intravenously and that reappearing in the urine (Table 35), it is seen that, in our experiments, the latter was only a small fraction of the former ranging between 3 per cent. in Experiment 2 and 25 per cent in Experiment 9. Thus the laked haemoglobin in the rabbit's plasma does not pass through the kidneys except in a relatively small amount, the greater part of the dissolved haemoglobin being dealt with in the body. We have here a striking parallel with the effect of injecting egg albumin into animals. In the latter case the foreign proteid injected is, in carnivora, broken up in the body, the nitrogen it contains being eliminated as urea in the urine, and only a small fraction escaping through the kidneys unchanged.\*

In the experiments recorded in Table 35 repeated examinations of the blood plasma were made so that it becomes possible to study the rate at which elimination of dissolved haemoglobin has taken place. This proceeded rapidly at first and subsequently progressed more slowly. In one Experiment (4, Table 35) a marked irregularity in the rate of disappearance occurred, the percentage of dissolved haemoglobin in the plasma remaining almost unchanged for two

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\* Friedemann and Isaac. *Zeitschr. f. exper. Pathologie*, 1905, Bd. 1, S. 513, quoted by v. Noorden, *Pathology of Metabolism*, London, 1907, Vol. 2, p. 91.

hours. The reaction rate formula for bimolecular and multimolecular reactions does not apply to these experiments. The rate of disappearance of haemoglobin follows, however, fairly closely a monomolecular course, as the values of  $K$ , calculated in the fourth column of Table 37, show. This will be further evident when the percentages calculated in the last column of this table are compared with those determined by experiment, in the third column. It thus becomes possible to calculate the haemoglobinaemia present immediately after injection, and from this the total volume of blood in the animals at the beginning of the experiment can be determined (Col. 3, Table 35). When the dissolved haemoglobin has been reduced to less than 10 per cent. of its original amount, further destruction proceeds with considerable and rapidly increasing slowness. The rate of disappearance thus is similar to that observed in Table 17. Contrary to what would be expected there seems to be considerable difficulty in removing the last traces of haemoglobin from the plasma. It may here be observed that rabbits which have been injected with dissolved haemoglobin show small percentages of haemoglobin in their blood plasma for many days afterwards, in this respect resembling human beings and differing from normal rabbits. The curious change in the rate of disappearance of haemoglobin, exhibited in Experiment 4, in which the percentage of haemoglobin remained almost unaltered between the hundred and thirty-fourth and the two hundred and fifty-seventh minutes, is difficult to interpret. The rate of disappearance of haemoglobin is relatively slow in Experiments 2, 5 and 9, in which the value of  $K$  is 0.00105; in the other experiments  $K$  ranges between 0.0029 and 0.0056. These values stand, except in Experiment 9, in a definite relation to the age of the animal used for experiment; in Experiments 2, 5 and 9 the animals employed were young, and the total blood was, as already mentioned, about one-tenth of the body weight.

The question arises, does the disappearance of the dissolved haemoglobin depend upon decomposition occurring in the blood plasma? This enquiry cannot be directly settled by experiment in vitro with the non-oxalated plasma of the rabbit, owing to the rapidity with which clotting takes place. In Table 38 are recorded some observations made with oxalated plasma, and with plasma defibrinated by shaking with glass beads. In Experiment 1 the

TABLE 37. The rate of disappearance of dissolved haemoglobin from the blood plasma in the living body of the rabbit. Cp. Table 35.

No. of Experiment (Table 29)	<i>t</i>	Corresponding relative percentage of haemoglobin (found by experiment)	$\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$ *	Calculated relative percentage of haemoglobin
2 Weight of rabbit = 1083 g. Blood $\frac{1}{520}$ Body weight $\frac{1}{10}$	0 min. 10 " 170 " 520 " 1320 "	[100] 96 59 33 8	0.00116 ) Mean 0.00095 ) 0.00105 0.00084	[100] [K=0.00105] 96 68 27 4
3 Weight of rabbit = 1440 g. Blood $\frac{1}{218}$ Body weight $\frac{1}{14.5}$	0 " 11 " 36 " 98 " 166 " 218 " 279 " 476 " 1346 "	[100] 91 69 42 36 22 17 5 3	0.0031 ) 0.0045 ) 0.0038 ) Mean 0.0027 ) 0.0030 0.0030 0.0026 0.0028 0.0011	[100] [K=0.0030] 92 78 51 32 22 15 4 0.0009
4 Weight of rabbit = 1710 g. Blood $\frac{1}{257}$ Body weight $\frac{1}{15}$	0 " 27 " 73 " 134 " 206 " 257 " 334 " 448 " 1386 "	[100] 78 69 27 26 25 16 10 2	0.0041 ) 0.0022 ) 0.0042 ) Mean 0.0028 ) 0.0029 0.0023 0.0024 0.0021 0.0012	100 [K=0.0029] 81 62 43 26 18 11 4 0.01
5 Weight of rabbit = 1025 g. Blood $\frac{1}{45}$ Body weight $\frac{1}{11}$	0 " 21 " 45 " 85 "	[100] 96 92 73	0.00081 ) 0.00075 ) Mean 0.00160 ) 0.00105	100 95 [K=0.00105] 90 81
6 Weight of rabbit = 1710 g. Blood $\frac{1}{180}$ Body weight $\frac{1}{15}$	0 " 8 " 62 " 117 " 180 " 237 " 297 " 358 "	[100] 94 59 38 31 19 15 10	0.0031 ) 0.0037 ) 0.0036 ) 0.0030 ) Mean 0.0024 ) 0.00305 0.0028 0.0028	100 94 [K=0.00305] 64 44 28 19 12 8
7 Weight of rabbit = 912 g. Blood $\frac{1}{206}$ Body weight $\frac{1}{12}$	0 " 6 " 151 " 33 " 206 " 544 " 67 "	[100] 96 36 86 38 8 —	0.0029 0.0020 ) Mean 0.0020 ) 0.0020	100 86 [K=0.0020] 39 8 —
8 Weight of rabbit = 1210 g. Blood $\frac{1}{172}$ Body weight $\frac{1}{15}$	0 " 17 " 62 " 88 " 118 " 152 " 172 " 315 " 383 " 442 " 498 "	[100] 89 55 47 44 38 29 21 16 13 11	0.0058 ) 0.0039 ) 0.0032 ) 0.0027 ) Mean 0.0031 ) 0.0030 0.0021 0.0020 0.0020 0.0019	[100] [K=0.0030] 89 58 54 44 35 30 11 7 5 1
9 Weight of rabbit = 1710 g. Blood $\frac{1}{168}$	0 " 13 " 168 "	[100] 99 70	0.0009	100 99 70
10 Weight of rabbit = 1760 g. Blood $\frac{1}{79}$ Body weight $\frac{1}{20}$	0 " 5 " 41 " 79 " 132 "	[100] 94 56 32 27	0.0062 ) Mean 0.0062 ) 0.0056 0.0042	100 [K=0.0056] 94 56 36 18

\* In Experiments 2, 7, 9 and 10 the formula  $\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$  is employed

haemoglobin and plasma employed were those of Rabbit 5, Tables 35 and 37; in Experiment 3, those of Rabbit 6, Tables 35 and 37. In all three experiments the disappearance of laked haemoglobin took place very slowly, compared with that occurring in the living body, less than 30 per cent. being broken up in six and a half hours. No data are available to indicate the rate at which in the circulating blood, destruction is effected solely by the plasma, nor do our experiments permit any conclusion to be drawn as to the part played by the body tissues in the removal of haemoglobin. The disappearance of the dissolved haemoglobin is also, to a small extent, affected by the action of the kidneys. To what extent this affects the percentage present in the blood plasma cannot be precisely determined, since it is unknown how far the amount of water leaving the blood to pass through the kidneys is replaced by water absorbed from the contents of the stomach and intestines.

#### SUMMARY

The principal points in the foregoing may be summed up as follows:—

1. In oxalated blood plasma obtained from healthy individuals, 0.10 per cent., or less, to as much as 0.25 per cent. of dissolved haemoglobin was found; in oxalated blood plasma obtained during black-water fever, while the urine in the bladder still contained haemoglobin, the amount of dissolved haemoglobin was usually, but not always, greater than this, ranging between 0.30 per cent. and 0.95 per cent.
2. Haemoglobinaemia produced experimentally in the rabbit is accompanied by haemoglobinuria, the percentage of haemoglobin in the urine quickly surpassing that in the blood plasma (Experiments 8-10, Table 35), and subsequently falling as the latter diminished.
3. The disappearance of dissolved haemoglobin from the blood plasma proceeds in the living body of the rabbit at approximately the same rate as a monomolecular chemical reaction.



TABLE 38. Decomposition of dissolved haemoglobin in oxalated or defibrinated plasma. Temperature of experiment 37° C.

No. of Experiment	Source of plasma and haemoglobin	Percentage of plasma	Percentage of 0.9% NaCl	Percentage of laked haemoglobin		Haemoglobin remaining at end of experiment	Remarks
				at beginning of experiment	at end of minutes		
1	Rabbit ...	80% (oxalated)	13.44%	6.56% at beginning of experiment	4.64% at end of 395 minutes	70.7%	Solution of laked haemoglobin added to plasma
2	Goat ...	Undiluted (defibrinated)	Nil	2.88% at beginning of experiment	2.36% at end of 480 minutes	82.0%	Haemoglobin dissolved during defibrination
3	Rabbit ...	Undiluted (defibrinated)	Nil	4.80% at beginning of experiment	4.14% at end of 300 minutes	86.2%	Haemoglobin dissolved during defibrination

#### IV. MECHANISM OF PRODUCTION OF SUPPRESSION OF URINE IN BLACKWATER FEVER.

With a view of throwing light upon the mechanism of production of blackwater fever, the action of quinine on red cells, the relation between haemolysinaemia and blackwater, and that existing between haemoglobinaemia and haemoglobinuria, have been studied in the preceding sections. Before proceeding to consider (in the next section) how far the data obtained in the last section are capable of accounting for the haemoglobinuria of blackwater fever, it will be convenient to deal, first of all, with a not uncommon sequel of blackwater, namely, suppression of urine, since it may readily be surmised that the mechanism of production of this condition is closely linked up with that of the haemoglobinuria of blackwater fever. The investigation of the mechanism of suppression of urine in blackwater fever is in its turn intimately associated with the study of another phenomenon not hitherto referred to, that is the occurrence of casts in the urine. In the cases of blackwater fever which came under our observation, these were met with not only during the period of haemoglobinuria and for a short time after its cessation, but also during suppression, when only a very small amount of amber-coloured urine was being passed. Casts were also present during the haemoglobinuria experimentally produced in rabbits, already described in the preceding section. For convenience of description, it will be better to take first the appearance of the suspended matter in the urine in experimental haemoglobinuria; then that of the suspended matter in the urine in blackwater fever; after which the condition of the kidneys in the latter condition will be described, and lastly the bearing of the data thus obtained upon the mechanism of production of suppression of urine will be considered.

The urine of all the rabbits in which haemoglobinuria had been produced experimentally (Table 35) contained, with one exception, solid matter at the time the urine was voided. The urine was at this time free from the ordinary crystalline or amorphous deposit found in urine, especially upon standing, and the solid matter which it contained (cp. Table 39) consisted of casts containing granules of a more or less dark colour, scattered sometimes in very small numbers

and irregularly (Figs. 22, 24), sometimes very densely packed together (Figs. 23, 24), the casts then becoming of a dark brown or reddish brown, almost black, colour. The size of the granules varied from  $0.5 \mu$ , or less, to as much as  $4 \mu$  to  $4.5 \mu$ , not infrequently both

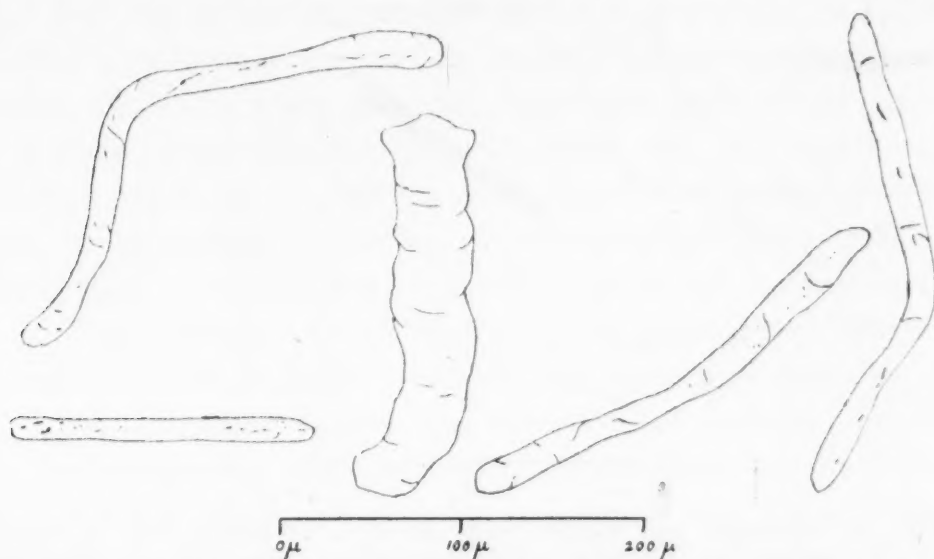


FIG. 21. Hyaline casts in urine of rabbit (Exp. 2, Table 35) four hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

fine and coarse granules being present together (Figs. 22 and 24). Free granules were also met with, sometimes, if not generally, obviously due to the breaking up of the casts. The free granules appeared in the form of masses, held together by colourless material

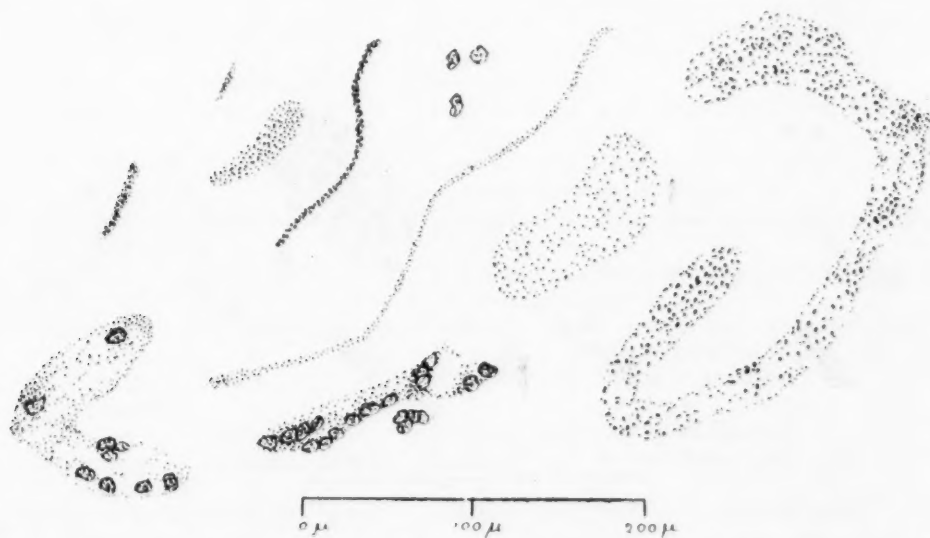


FIG. 22. Granular and epithelial casts in urine of rabbit (Exp. 3, Table 35) twenty hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

of a very soft consistence; sometimes they appeared to be quite unconnected with one another. The granules occasionally on standing became partly obliterated as the result of decomposition of the urine. When this occurred, as also when the granules were

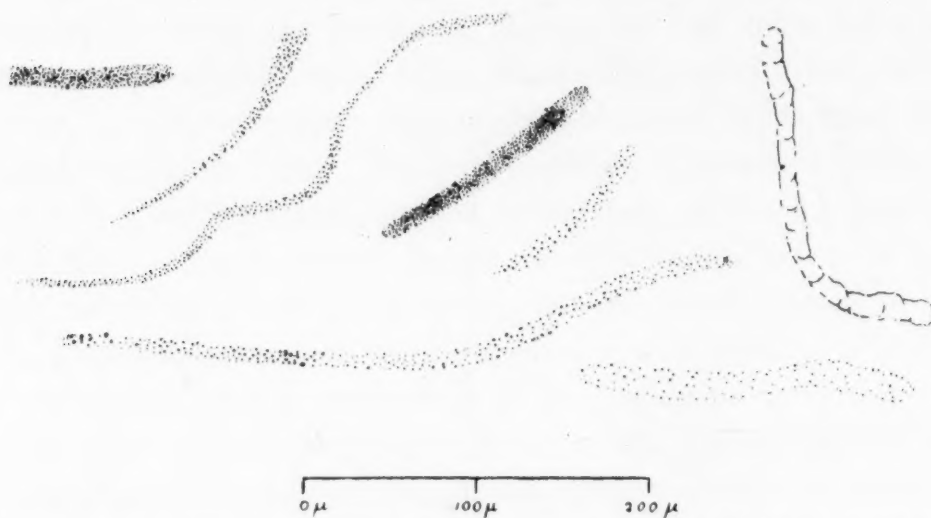


FIG. 23. Granular and hyaline casts in urine of rabbit (Exp. 5, Table 35) eighty-five minutes after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

scanty, it became possible to observe the character of the matrix of the casts. This was of a hyaline material, not unfrequently showing creases or bends (Figs. 21, 23). Sometimes the casts exhibited here and there small masses suggestive of nuclei. In the unstained condition it was difficult to decide whether these were really nuclei or

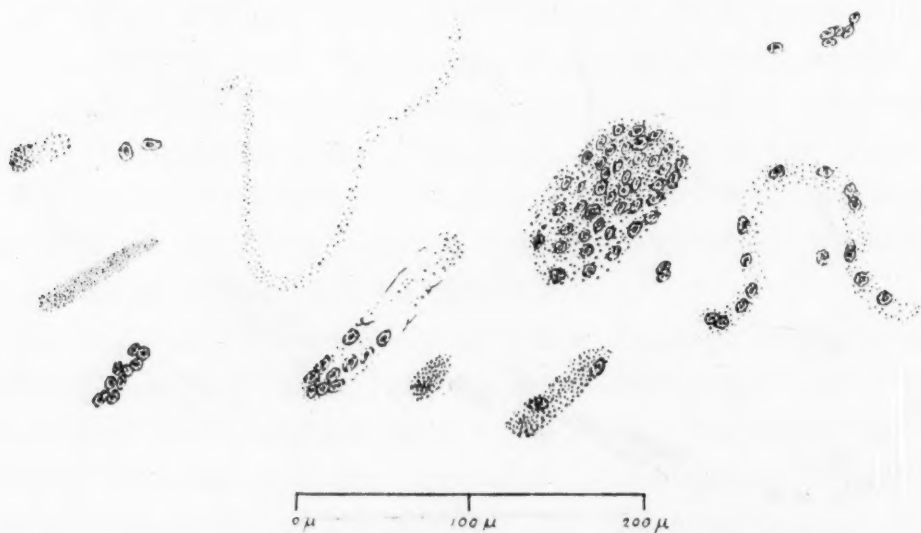


FIG. 24. Granular and epithelial casts in urine of rabbit (Exp. 6, Table 35) twelve and a half hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.



not, but when a little methylene blue solution was added these masses could be recognised to be nuclei resembling those of the epithelial cells of the renal tubule, from which they obviously arose. The casts varied considerably in length, being from  $20\mu$  to as much as  $400\mu$  long, their diameter ranging from  $3\mu$  to  $30\mu$ , or more (Figs. 21 to 24). The size of the degenerated nuclei ranged from  $7\mu$  to  $12\mu$ . The nuclei were usually very few in number, but occasionally became numerous, the casts then becoming epithelial casts (Figs 22, 24). Free epithelial cells with degenerated nuclei were also found, sometimes singly, sometimes in groups of two and three, in the latter case forming a transitional condition between single free cells and epithelial casts. All the free cells met with in the haemoglobinous urine of Experiments 1 to 10, Tables 35 to 39, were of spheroidal form, and appeared to be derived from the uriniferous tubules. The urine, when examined before the injection of haemoglobin, was always free from cells. The amount of solid matter contained in the haemoglobinous urine in these experiments was very variable (Table 39), being sometimes very small, sometimes abundant, forming on standing a precipitate of as much as a sixth of a column. This variation appeared in Experiments 9 and 10 (Figs. 8 and 9) to be

TABLE 39. Characters of deposit from urine of rabbit during haemoglobinuria experimentally induced. Cp. Tables 35 and 37.

No of Experiment	Colour	Amount	Microscopic characters
1	Brown	Very small	No casts
2	Brown	Very small	Hyaline casts with here and there fine granules (Fig. 21)
3	Greyish black	Very small	Granular casts (large and fine granules), very few renal epithelial cells (Fig. 22)
4	Chocolate coloured	Abundant ( $\frac{1}{20}$ col.)	Granular casts (large and fine granules) and granular debris
5	—	Very small	Granular casts (large and fine granules) and hyaline casts (Fig. 23)
6	Chocolate coloured	Abundant ( $\frac{1}{30}$ col.)	Granular casts (coarse and fine granules), free granules, renal epithelial casts (Fig. 24)
7	Chocolate coloured	Small	Granular casts and free granules
8	—	Small	Granular and renal epithelial casts
9	Chocolate coloured	Abundant ( $\frac{1}{8}$ col.)	Granular casts and renal epithelial cells
10	Chocolate coloured	Abundant ( $\frac{1}{8}$ col.)	Granular casts and renal epithelial cells

related to the percentage of haemoglobin in the urine, being most abundant when the latter was highest, but further experiments are required to settle this point definitely. The deposit from the urine on centrifugalisation was always dark in colour, sometimes of a dark chocolate colour, sometimes of a greenish-black colour. Red cells were not met with. The deposit diminished in amount as the haemoglobinuria lessened, but was still present for about a day after the haemoglobinuria had ended.

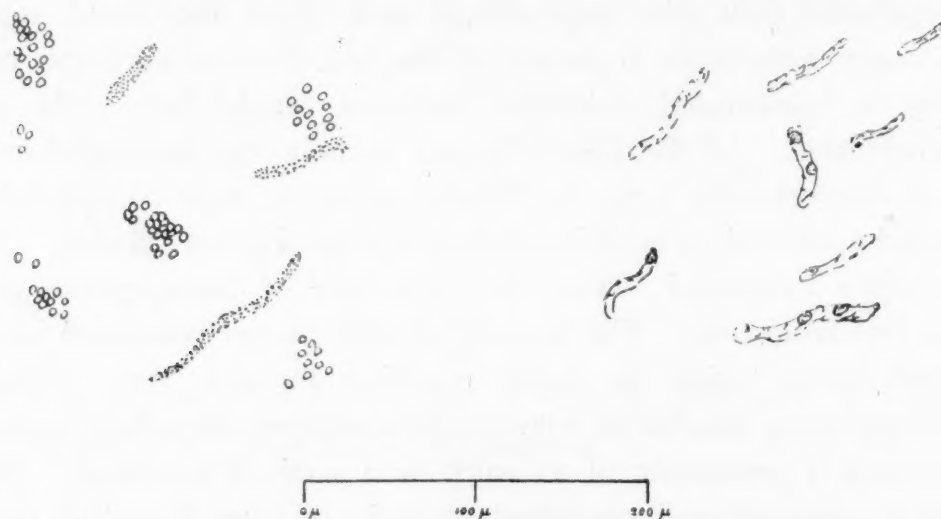


FIG. 25. To left, deposit from chocolate-coloured urine of Case 2 (second day), consisting (in addition to pus cells and bacteria, not sketched) of red cells, more or less decolourised, together with a few granular casts. To right, deposit from dark amber-coloured urine of Case 5 (fifth day), consisting of hyaline casts, in which a few indistinct nuclei can be seen. Magnification 180 diameters.

Similar casts and free epithelial cells were also found in the chocolate coloured deposit of the urine during redwater in cattle and dogs, due to piroplasmosis.

The urine in the twenty attacks of blackwater fever studied, contained, in all cases in which this point was determined, that is in all except Cases 1 and 12, Table 40, solid matter chiefly made up of granular casts and granular debris, similar in appearance to that already described in experimental haemoglobinuria in rabbits. When unmixed with the crystalline and amorphous deposits formed in urine which has stood for some time, the suspended matter was found on centrifugalisation to be of a chocolate-brown colour. As in experimental haemoglobinuria, it varied considerably in amount in different cases, being sometimes scanty, sometimes when allowed to sediment

amounting to a fifth of a column of the urine. As in the former experiments, so here also it is not possible, from the data at present available, to state the conditions which determine the amount of suspended matter; no clear relationship between the amount of

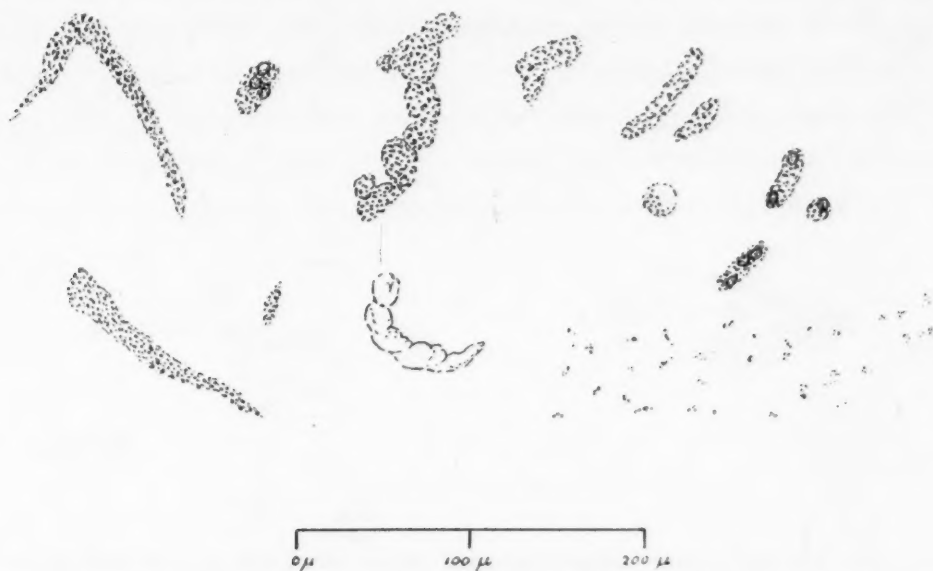


FIG. 26. Deposit from porter-coloured urine of Case 3 (second day). Granular casts and free granules are seen. Also one hyaline cast. Nuclei can be recognised in four of the casts. Magnification 180 diameters.

suspended matter, on the one hand, and the percentage or the total amount of haemoglobin eliminated in the urine, on the other, is recognisable by a comparison of Table 40 with Tables 43 and 45.

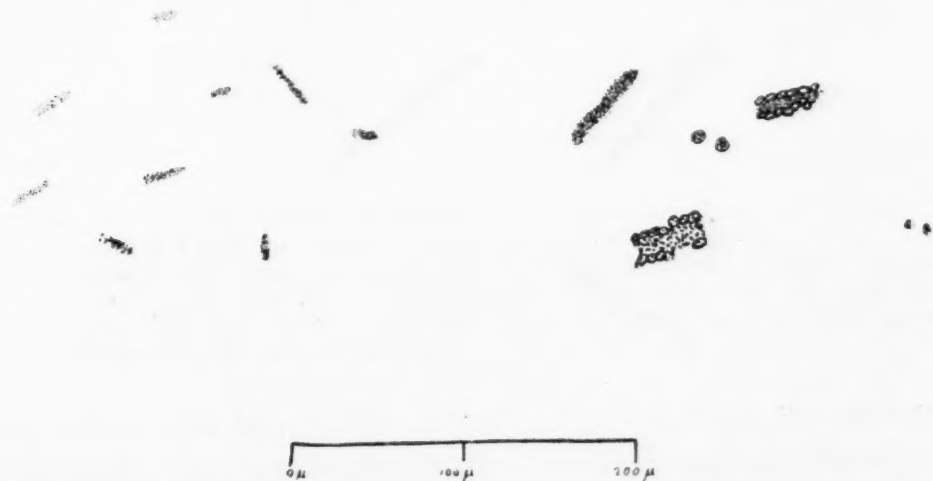


FIG. 27. To left, deposit from amber coloured urine of Case 4 (third day), consisting of small granular casts. To right, deposit from port wine-coloured urine of Case 8 (third day), showing three granular casts, two of which contain degenerated nuclei, derived from a renal tubule; two squamous cells are also seen. Magnification 180 diameters.

UO 11

The amount of deposit diminishes with the haemoglobinuria, but after the urine has remained free from haemoglobin as long as three days, casts may still be found in it.



FIG. 28. Deposit from porter-coloured urine of Case 7 (second day), consisting of granular casts, the granules being dense in the smaller casts. Magnification 180 diameters.

The appearance of the casts and granules in the urinary deposit in the blackwater fever cases examined (Figs. 25 to 31) presented slight points of difference from that seen in the rabbit's urine. The length of the casts varied from  $15\ \mu$  to  $200\ \mu$ , or more, their diameter

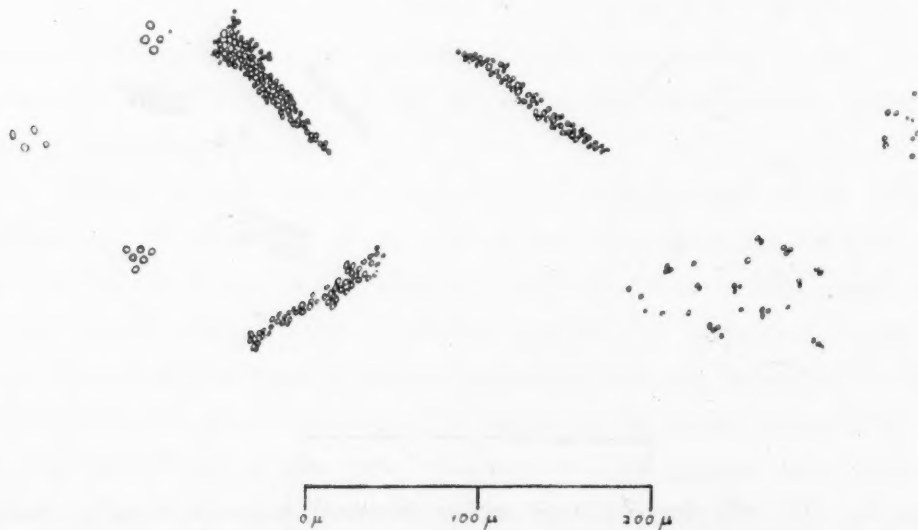


FIG. 29. Deposit from porter-coloured urine of Case 7a (first day), consisting of granular casts and debris, and (to left) a few red cells. The granules are of dark brown colour and large size, having a diameter of  $4\ \mu$  to  $4.5\ \mu$ . Magnification 180 diameters.



ranging between  $3\ \mu$  and  $25\ \mu$ , rarely more. The casts were brown in colour, the depth of colour depending upon the sparsity or abundance of the granules present. When the casts contained very few granules their hyaline matrix became visible (Figs. 25, 26). Sometimes the granules were scattered irregularly and in small numbers (Figs. 25, 28, 31), sometimes very densely throughout the casts (Fig. 30). The granules were sometimes very fine, not more than  $0.5\ \mu$  in diameter (Figs. 25, 28), sometimes very coarse, reaching  $4\ \mu$  in diameter (Fig. 29); intermediate forms were common (Figs. 26, 30).

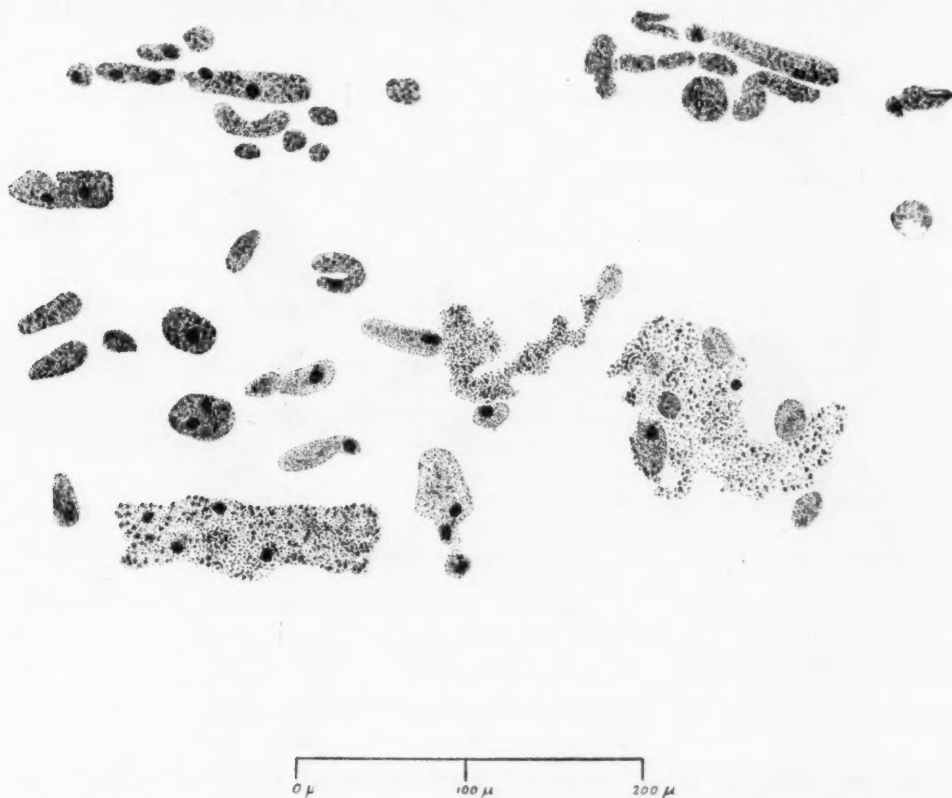


FIG. 30. Deposit from porter-coloured urine of Case 15 (third day), stained with a watery solution of methylenblue. The deposit consists of granular casts and masses, in some of which nuclei of renal origin are seen. The granules are mostly fine, but some coarse granules are seen. A few free renal cells are also present. Magnification 180 diameters.

The colour was brown, the largest granules being the darkest; but occasionally variation in the depth of colour was met with irrespective of size. Masses of granules were also met with, some obviously representing broken-down casts, as well as free granules (Fig. 29), as in haemoglobinuria of the rabbit. The consistence of the granular casts varied considerably, some being hard and dry (Figs. 27, 28, 30),

others being soft and presenting a swollen appearance (Figs. 26, 31); the former were sometimes small, the latter of large size, as the sketches indicate. Hyaline casts (Figs. 25, 26) were much less frequent than granular casts. Casts containing one or more nuclei (Figs. 25, 30, 31), and epithelial casts (Figs. 27, 30, 31), were not uncommon. Free cells and cell masses were also seen (Figs. 27, 30, 31); the cells were mononuclear, of spheroidal aspect, with sometimes moderate, sometimes abundant, cytoplasm, and obviously came from the uriniferous tubules. Red blood cells, which were not met with in the rabbit's urine during experimental haemoglobinuria, were

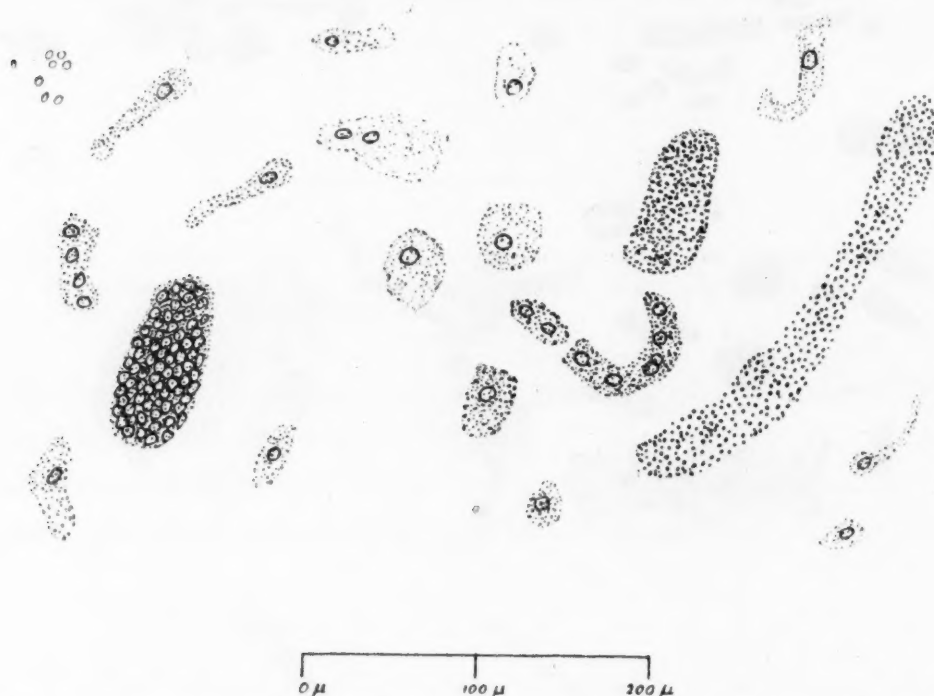


FIG. 31. Deposit from porter-coloured urine of Case 17 (second day), consisting of numerous epithelial cells; also in smaller numbers granular and epithelial casts and a few red blood cells, the latter being shown in the upper left hand corner of the sketch. Magnification 180 diameters.

encountered during the haemoglobinuria of blackwater fever in ten out of the twenty attacks investigated (Tables 40 and 43; Figs. 25, 29, 31). These were usually present in very small numbers, but in Case 2 (pp. 178 to 181) their number was considerable; this case will be referred to at length later (p. 149). In three cases (7a, 10, 11; cp. clinical notes, pp. 195, 207 and 217) red cells were not present at first, but as the haemoglobinuria was passing off made their appearance. All the above formed elements disappeared completely within three

or four days after the disappearance of haemoglobinuria, except in Cases 7a and 11, in which suppression of urine occurred; in these casts or plugs continued for eight and five days respectively, until death occurred.

Occasionally squamous epithelial cells were met with, and sometimes also pus cells (Cases 2 and 9), the latter persisting after complete recovery from blackwater fever. Short bacilli were present in one case (Case 2), both before and after the attack. In no other case were bacteria seen, and in no case were organisms or cells other than those already described met with.

TABLE 40. Characters of deposits from urine during haemoglobinuria of blackwater fever.

Case	Colour	Amount	Microscopic characters
1	—	—	Red cells in very small numbers
2	Chocolate coloured	Abundant ( $\frac{1}{4}$ col.)	Numerous red blood cells, pus cells, granular casts, free granules, epithelial cells (Fig. 25)
3	Whitish	Abundant ( $\frac{1}{8}$ col.)	Granular casts and masses (fine granules), epithelial casts (on seventh day very few red cells) (Fig. 26)
4	—	Small	Granular casts and masses (Fig. 27)
5	Brownish	Small	Hyaline masses or casts (Fig. 25)
6	—	Small	Granular casts, renal epithelial cells, few red cells
6a	—	Small	Red cells, granular casts and masses, renal epithelial cells
7	Dark brown	Abundant ( $\frac{1}{8}$ col.)	Granular casts (fine granules) and masses, renal epithelial cells (Fig. 28)
7a	Dark brown	Abundant ( $\frac{1}{15}$ col.)	Granular casts (coarse granules), renal epithelial cells, and red cells (Fig. 29)
8	—	Small	Granular casts, few renal epithelial and red cells (Fig. 27)
9	Whitish	Small ( $\frac{1}{10}$ col.)	Pus cells, granular masses and casts (examined on eighth day)
10	—	Abundant ( $\frac{1}{10}$ col.)	Granular casts and masses, renal epithelial cells (on fourth day very few red cells)
11	—	Abundant	Granular casts and masses, renal epithelial cells (on fourth day very few red cells)
12	—	Abundant	Granular and hyaline casts, renal epithelial cells
13	—	—	—
14	—	Small	Granular and epithelial casts and renal epithelial cells
14a	Brownish white	Small ( $\frac{1}{10}$ col.)	Granular casts (coarse granules) epithelial casts
15	Brown	Small ( $\frac{1}{80}$ col.)	Granular, hyaline and epithelial casts, free granules (coarse and fine) (Fig. 30)
16	Whitish	Small	Granular casts, renal epithelial cells
17	Chocolate coloured	Abundant ( $\frac{1}{12}$ col.)	Granular and epithelial casts (coarse and fine granules) renal epithelial cells, few red cells (Fig. 31)

During suppression of urine in blackwater fever (Cases 7a and 11, Table 41) casts continued to be found in the urine. The amount

of deposit obtainable on standing or centrifugalisation was extremely small. In Case 7a it appeared of a brownish colour and in Case 11 of a whitish aspect, but its colour was difficult to estimate owing to the small amount obtainable; in the former case, for example, if a sufficient amount of deposit had been available, it would probably, judging from its microscopical characters, have been of a dark reddish brown or chocolate colour. The deposit consisted principally of dark granular casts, which, in Case 7a, were just visible to the naked eye, being about  $100\ \mu$  to  $150\ \mu$  long and  $40\ \mu$  to  $60\ \mu$  broad.

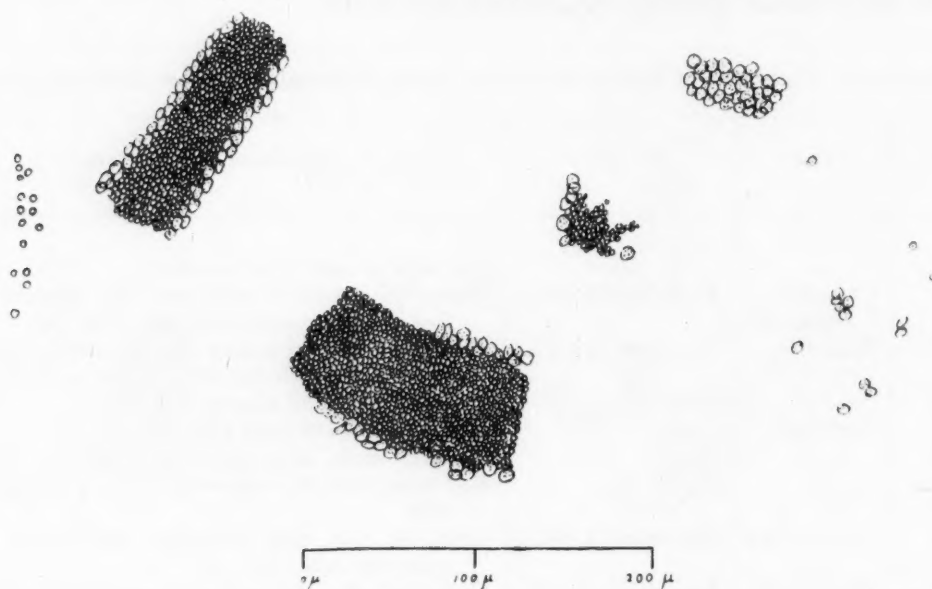


FIG. 32. Deposit from light yellow-coloured urine of Case 7a (third day, during suppression of urine). On the left are a few red blood cells. In the middle are two large casts and a fragment of a third, containing large coarse granules, closely packed and bounded externally by epithelium derived from the collecting tubules. To the right are renal epithelial cells and cell masses. Cp. with Figs. 28 and 29. Magnification 180 diameters.

The granules were of more or less spherical form, very coarse, having a diameter of about  $5\ \mu$ , of a dark reddish brown colour, in their aggregated condition somewhat resembling blood clot, but of a deeper colour, and were densely packed together, so that it was sometimes difficult to discern the outlines of the individual granules (Fig. 32). No fine or medium sized granules were observed. The casts were covered externally with a layer of epithelium, and from their size and the appearance of the epithelium it is clear that most of these casts were formed in the ducts of Bertini which they completely plugged. In addition, masses of epithelial cells and single



epithelial cells, both presumably of renal origin and apparently separated off from the casts, were met with, as also red cells in small numbers in both Case 7a (Fig. 32) and Case 11. Casts and red blood cells continued in the urine during the whole period intervening between cessation of haemoglobinuria and death. No bacteria or other formed elements were met with; hyaline casts were absent. The amount of the deposit, which could not be accurately measured, owing to the small amount of urine passed, but was relatively as well as actually extremely small, did not appear to vary much from day to day during the period of suppression, nor did the composition of the deposit show any marked change during this period.

TABLE 41. Characters of deposit from urine during suppression in blackwater fever (Cases 7a and 11).  
Case 16 is also given in this Table.

Case	Colour	Amount	Microscopical characters	Coagulable proteid
7a—2nd day	Brownish	[See p. 199] very slight	Red cells, granular casts (coarse granules) surrounded by renal tubule cells, granular debris, free renal cells. [Fig. 32]	Precipitate $\frac{1}{4}$ col. to $\frac{3}{4}$ col., of slightly brownish white colour
" 3rd "	"	[Urine 21 c.c.] "		
" 4th "	"	[Urine 11 c.c.] "		
" 5th "	"	[Urine 17 c.c.] "		
" 6th "	"	[Urine 33 c.c.] "		
" 7th "	"	[Urine 48 c.c.] "		
" 8th "	"	[Urine 35 c.c.] "		
" 9th "	"	[Urine 23 c.c.] "		
" 10th "	"	[Urine 43 c.c.] "		
11—4th "	Nearly white	[Urine 48 c.c.] slight	Granular casts, epithelial cells, very few red cells	Precipitate $\frac{2}{3}$ col. to $\frac{1}{2}$ col. of white colour
" 5th "	"	[Urine 92 c.c.] "		
" 6th "	"	[Urine 73 c.c.] "		
" 7th "	"	[Urine 93 c.c.] "		
" 8th "	"	[Urine 85 c.c.] "		
" 9th "	"	[Urine 8 c.c.] "		
16—4th "	—	[Urine 336 c.c.] very slight	[On 2nd and 3rd days, deposit small, consisting of granular casts and epithelial cells]	$\frac{1}{10}$ col. white precipitate [sp. gr. 1.015]
" 5th "	—	[Urine 544 c.c.] "	—	" " "
" 6th "	—	[Urine 500 c.c.] "	—	—
" 7th "	—	[Urine 1400 c.c.] "	—	—
" 8th "	—	[Urine 840 c.c.] "	—	—
" 9th "	—	[Urine 420 c.c.] "	—	—

It will be noticed that the chief alteration in the appearance of the casts observed during suppression of urine consisted in their large size, in the coarseness of the granules they contained, and in the frequent presence of an external epithelial envelope.

Suppression of urine in blackwater fever is known not to be necessarily complete, and hence the term oliguria is sometimes employed to denote this condition, instead of the more familiar terms 'suppression' or 'anuria.' A search of the literature has not enabled us to find exact records of the amounts of urine passed in individual cases during suppression, but two of our own cases (7a and 11) furnish data on this point. The daily amounts in these two cases are given in Table 41, and will be seen to range in Case 7a from 11 c.cm. to 43 c.cm., and in Case 11 from 8 c.cm. to 92 c.cm. The urine was voided naturally, but owing to the small quantities present in the bladder some difficulty was experienced in voiding it, and it is not certain that the bladder was always completely emptied. In any case, however, the daily variations were not usually considerable. The average amount of urine secreted by Case 7a was 28 c.cm. per day, and by Case 11 66 c.cm. per day (Table 42).

TABLE 42. Average daily amount of urine passed during suppression of urine in blackwater fever. The colour of the urine was light yellow or amber.

Case	Period of suppression	Average amount of urine daily during suppression	Sp. gr. of urine	Coagulable proteid in urine
7a	Nine days	28 c.c.	1.010 to 1.015	$\frac{1}{4}$ col. to $\frac{2}{3}$ col.
11	Six days	66 c.c.	1.008 to 1.009 (on fourth and fifth days)	$\frac{2}{5}$ col. to $\frac{1}{2}$ col.

The urine was pale yellow or amber in colour, and was of markedly low specific gravity, namely, 1.010 to 1.015 in the first case and 1.008 to 1.009 in the second case. Unfortunately, no estimation of the amount of urea present could be made. Another striking feature of the urine was the large amount of coagulated proteid which it contained, the precipitate produced on acidifying and boiling measuring, on standing,  $\frac{1}{4}$  col. to  $\frac{2}{3}$  col. of the urine taken. In Table 41, together with the above two cases, in which death occurred on the tenth and ninth days respectively, another case is also recorded, because the question arose whether this case, in which death occurred on the ninth day, should be regarded as one of partial suppression. The average daily amount of urine was 640 c.cm. ( $22\frac{1}{2}$  oz.), and its

specific gravity was somewhat low, being 1.015. Unfortunately, as in the preceding cases, no estimation of the output of urea was possible. The coagulable proteid obtained on acidifying and boiling measured, on standing,  $\frac{1}{10}$  col. Further reference will be made to this case later.

Attention must now be directed to the condition of the kidneys at the time of death from suppression of urine in Cases 7a and 11. Before proceeding further, however, reference may be made to the literature of this subject. The number of post-mortem records of the aspect of the kidneys in suppression of urine in blackwater fever, which are accompanied by a report of the microscopical appearances of these organs, is very small. Two cases of death with suppression of urine in blackwater fever are described by H. Werner,\* death in one case (Case 1) taking place on the day of the attack, and in the other case (Case 3) on the third day after the commencement of haemoglobinuria. In the first case the kidneys were dark violet in colour and were not markedly enlarged. The medulla showed a dark brown striation to the naked eye. On microscopical examination a finely granular exudation was seen within the Malpighian capsules, and occasionally separated epithelial cells were noted, but the glomerular cells were unaltered. The epithelium of the convoluted and straight tubules was normal, but their lumen was enlarged and contained coagulated material exhibiting granules, which were fine near the glomerulus but became progressively coarser as the collecting tubules were approached. No trace of an inflammatory process in the connective tissue of the kidney was observed. Inside the blood vessels was seen black malarial pigment, and in some places plasmodial division forms. In the second case described by Werner the kidneys were of a pale brown to yellowish red colour, and were enlarged. The microscopical appearances were the same as in the first case, except that the epithelium of the convoluted tubules showed in places cloudy swelling.

The cases coming under our own observation were two in number (Cases 7a and 11, Tables 41 and 42; clinical histories, pp. 195 to 201 and pp. 217 to 222), death occurring, as already mentioned, on the tenth and ninth days respectively, the duration of suppression being

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\* Über die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- und Tropenhygiene, 1907, B. 11, S. 5.



eight days in the first case and five days in the second. The kidneys showed no obvious congestion, though somewhat dark in aspect. They were of a brownish colour. The capsule stripped readily in Case 7a, and with slight erosions in Case 11. A most striking feature of the kidneys was their marked enlargement, measuring in Case 7a

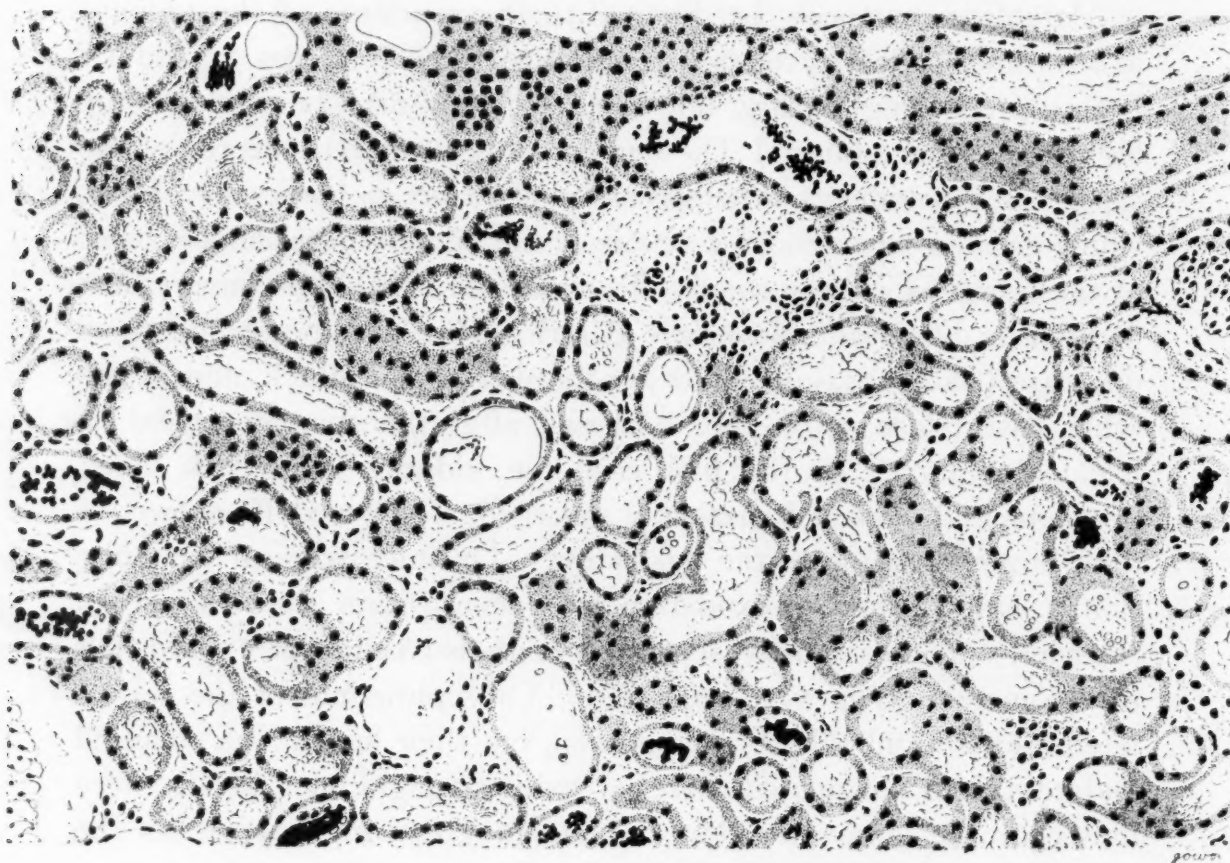


FIG. 33. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. The renal tubules are considerably distended with fluid. The lumen of thirteen of the tubules is partly occupied by darkly staining coarse granules measuring about  $5\mu$  in diameter, some of which are discrete, while others have coalesced into irregular masses. The epithelium of the tubules is flattened, but is not degenerated. The Malpighian capsules are dilated, but otherwise unaltered. The interstitial tissue, which seems to be also distended with fluid, exhibits no cell infiltration. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 120$ .

5in. in length by 3in. in breadth and  $1\frac{1}{4}$ in. in thickness; in Case 11 the enlargement was very nearly as great. The cortex could not be sharply differentiated from the medulla, so that it was not possible to say how far the degree of swelling was greater in the former as



compared with the latter. A further characteristic alteration in the aspect of the kidney was presented by the medulla, which was quite abnormal, being dark brown almost black in colour, especially at the bases of the pyramids. On closer examination it was seen that this was due to fine brown radiating lines, presenting a somewhat fan-like character and readily seen with the naked eye. Fine brownish dots could also be recognised, though not so readily, scattered here and

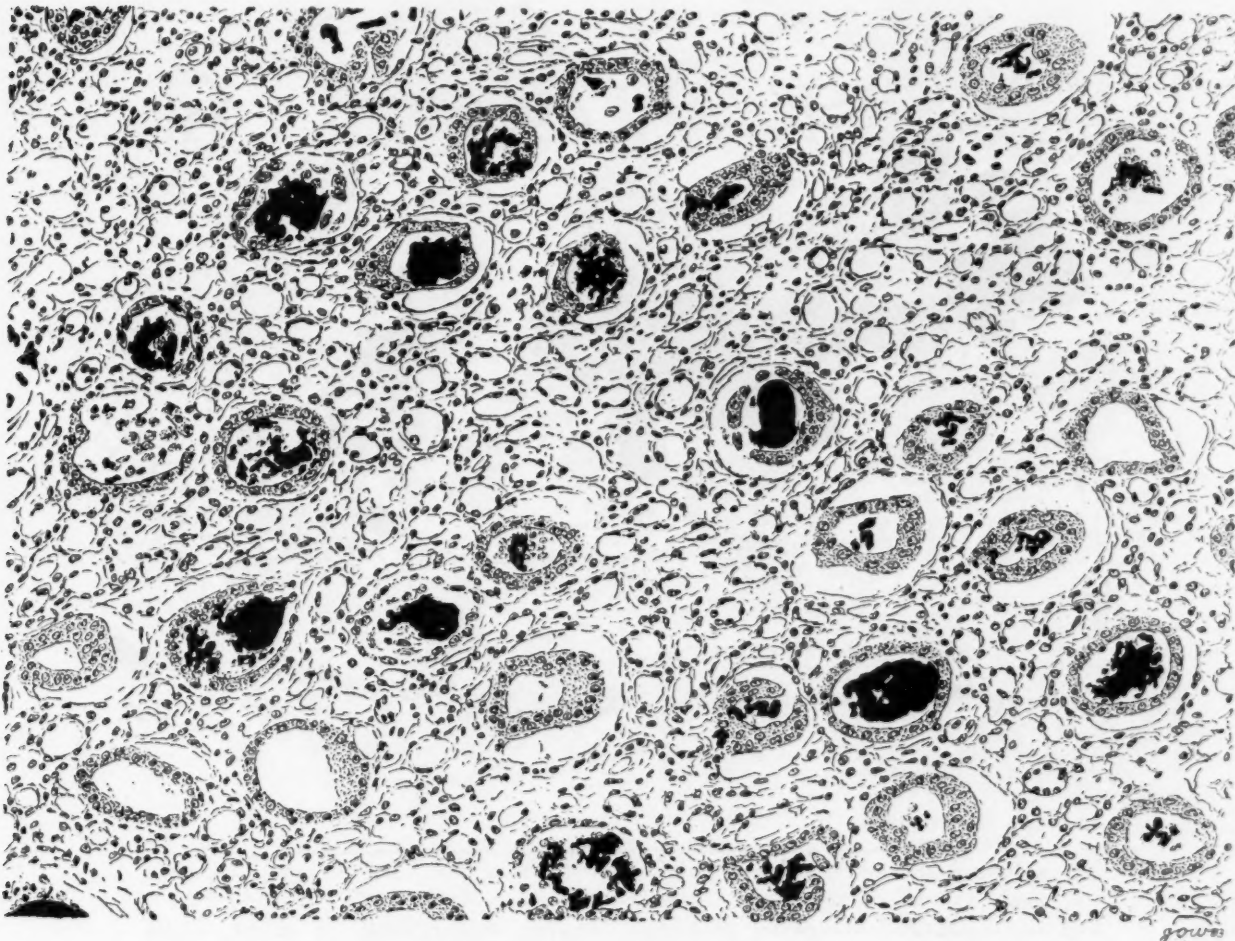


FIG. 34. Transverse section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. Many of the large collecting tubules, which are dilated and exhibit flattening of the epithelial lining, are partly filled with darkly staining granular material. The individual granules, which are about  $5\mu$  in diameter, are in some places discrete but are more frequently closely packed so as to form irregular masses. In some of the tubules also free epithelial cells are seen. The renal epithelium though flattened shows no degenerative changes. The interstitial tissue does not exhibit any cell infiltration or other marked alteration. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 130$ .

there throughout the cortex. The above two changes—the enlargement of the kidney and the distribution of the dark brown medullary striation and cortical stippling—are shown somewhat diagrammatically in Fig. 61, C. Recent haemorrhages of a punctate character were seen in Case 7a in the mucous membrane of the renal pelvis, as well as an ecchymosis about 1 cm. in diameter beneath the capsule of the left kidney.

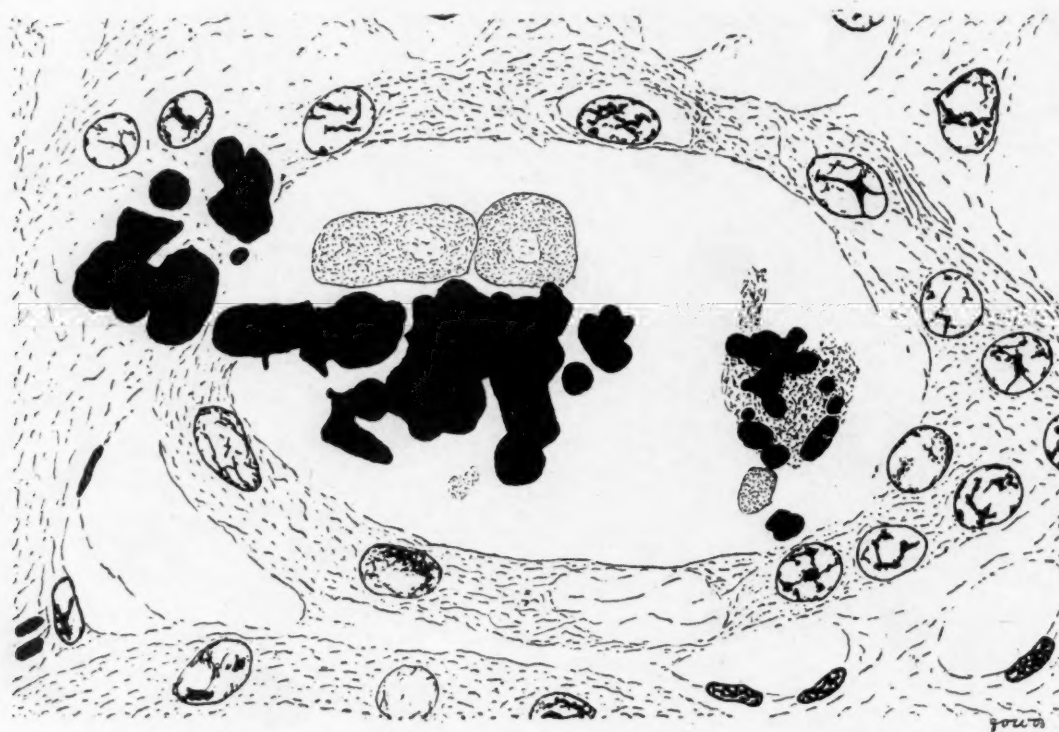


FIG. 35. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A dilated convoluted tubule is seen in transverse section. The lumen of the tubule is partly occupied by irregular masses, composed of darkly stained granules. The individual granules, when discrete, range from  $2\mu$  or  $3\mu$  to  $5\mu$  in diameter. To the right some finely granular debris is seen. Within the lumen of the tubule are two free epithelial cells, the nuclei of which are indistinct. The epithelium of the wall of the tubule is flattened, but does not present any marked evidence of degeneration. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 1350$ .

On making sections of the kidneys in Cases 7a and 11, it was found (Figs. 34, 36, 38, 40, 54 and 56)\* that many, but not all, of the

\* Figs. 53–60 are opposite p. 131 (Plates 1 and 2).

ducts of Bertini, the largest collecting tubules, contained dark reddish brown granular plugs  $40\mu$  to  $80\mu$  in transverse measurement, similar to those already described in the urine (Fig. 32), and giving rise to the brown radiation, already mentioned, visible to the naked eye

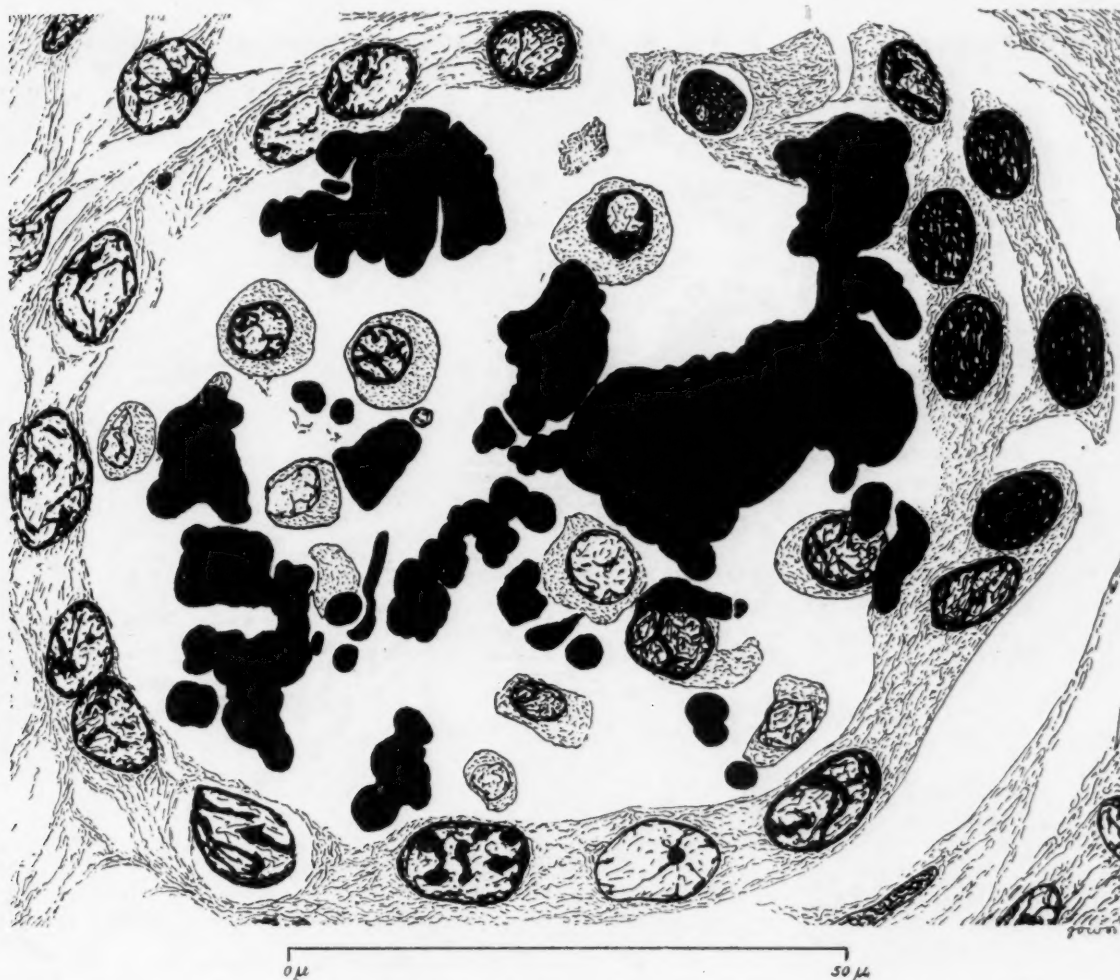


FIG. 36. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A collecting tubule is shown in transverse section. The lumen of the tubule is partly filled with free epithelial cells and irregular masses composed of darkly stained granules of varying size, which have for the most part coalesced. The size of the individual granules ranges from  $3\mu$  to  $5\mu$ . Some of the free epithelial cells contained within the tubule exhibit a well stained nucleus, the cytoplasm of the cell not being markedly altered in appearance; in others the cytoplasm is stained more darkly than usual and the nuclei are indistinct. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 1150$ .

in the medulla of the kidney. In places these plugs had been detached, sometimes, but not always, together with the epithelial lining of the collecting tubule, the basement membrane being then bare. In some cases a granular plug, covered externally with



epithelium, had been detached at a higher level and was found partly filling a duct of Bertini. In the smaller straight tubules similar plugs of lesser dimensions, from  $30\mu$  to  $40\mu$  in diameter, were frequently seen. In the uriniferous tubules (Figs. 33, 35, 37, 39, 53 and 55) granular plugs or casts were sometimes present, frequently absent.

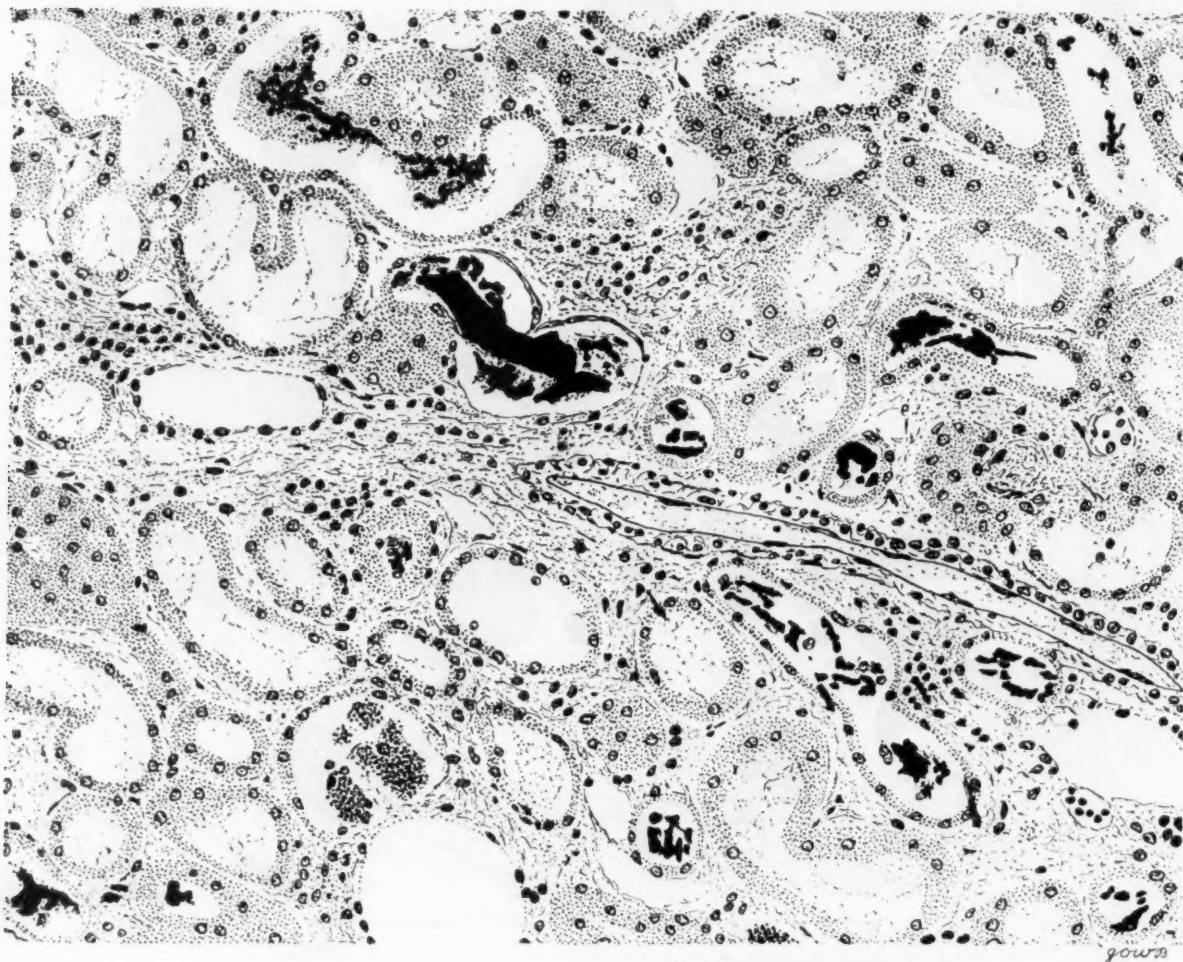
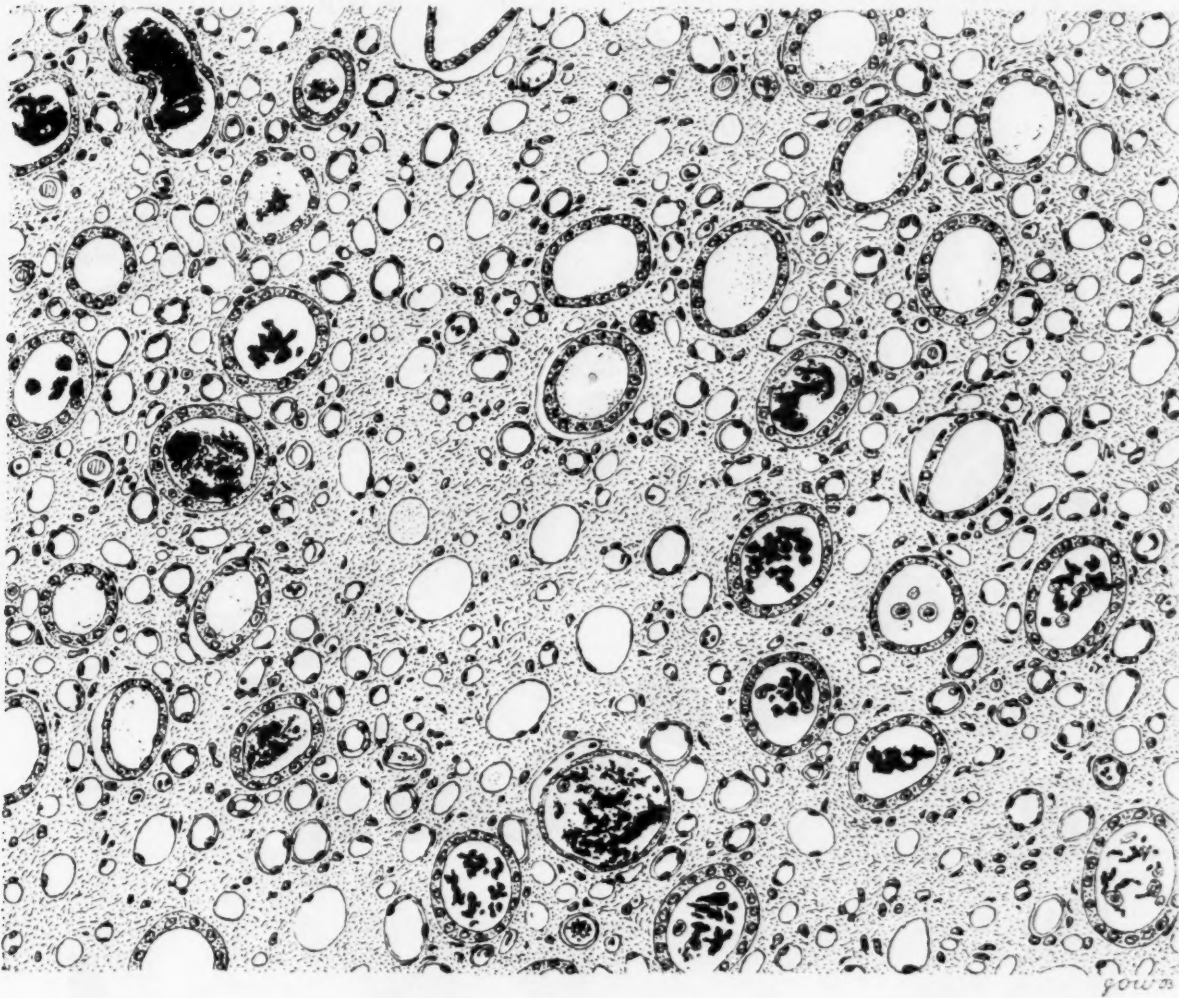


FIG. 37. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. The renal tubules are generally dilated to a considerable degree, the epithelium being in many places correspondingly thinned. In thirteen of the tubules dense black material is seen partly filling the lumen. In two situations this material is observed to consist of more or less discrete granules, while elsewhere the individual granules are indistinct. A small amount of flocculent material, with here and there a nucleus, can be recognised in some of the tubules. In one tubule the epithelium has disappeared from part of the wall of the tubule. The renal epithelium does not appear to be degenerated. The interstitial tissue is distended with fluid, but does not exhibit any cell infiltration. The blood vessels are not congested. Few red cells are seen. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 150$ .



These plugs, which varied considerably in diameter, ranging from  $30\mu$  to  $80\mu$ , gave rise to the brown stippling sometimes seen on a cut surface of the cortex of the kidney (Fig. 61).



0  $\mu$  150  $\mu$  300  $\mu$

FIG. 38. Transverse section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. About half of the collecting tubules are partly occupied by darkly staining material, which in some places appears uniform in aspect, but elsewhere when not very dense is seen to be made up of granules of varying size. In addition epithelial cells can be recognised in six of the collecting tubules. The collecting tubules are more or less distended and their epithelial lining is correspondingly thinned. The epithelium of none of the collecting tubules has been shed. The renal epithelium does not exhibit degenerative changes. The blood capillaries are with very few exceptions empty, no red cells being seen. The interstitial tissue is not markedly altered in appearance. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 155$ .

The renal plugs were seen in sections stained with haematoxylin to be very irregular and heterogeneous in aspect, not as a rule completely filling the lumen of the tubule. They were in part made up of granules, readily taking up the dye so as to be black in colour, and of irregular masses, measuring, as much as  $50\mu$  or more across, formed by the coalescence of the granules. In addition to these granules other elements were often present, namely, detached epithelial cells and smaller lightly stained granules, together with flocculent matter.



FIG. 39. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. A dilated renal tubule is seen in transverse section. The lumen of the tubule is partly occupied by darkly stained granules, varying in diameter from  $3\mu$  to about  $5\mu$ , which have joined together so as to form irregular more or less elongated masses. In addition one free epithelial cell is seen among the granular masses. The epithelial lining of the tubule can be recognised without difficulty, it is thinned, especially above. Except for this thinning, the result of dilation of the tubule, the epithelium does not seem otherwise altered. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 1300$ .



FIG. 40. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. Transverse section of a collecting tubule. The lumen of the tubule is incompletely filled with epithelial cells and darkly stained granular material, the individual granules, which are from  $3\mu$  to  $5\mu$  in diameter, having coalesced so as to form irregular groups. The nuclei of two of the free epithelial cells, contained in the lumen of the tubule present a shrunken aspect and do not exhibit a well formed chromatin network. The epithelial cells lining the tubule do not exhibit any marked change, and the interstitial tissue is free from any indication of cell infiltration. Hardened in alcohol. Stained with iron alum haematoxylin  $\times 1300$ .



The individual darkly stained granules were usually of large size, measuring  $3\mu$  to  $5\mu$  in diameter. They were of the same size in the cortex as in the medulla, not presenting any increase in size as the renal pelvis was approached. A few tubules were met with, both in the cortex and in the medulla, containing much smaller granules, measuring from  $2\mu$  to less than  $0.5\mu$  in diameter (cp. Fig. 37). These were sometimes very dark, almost black in colour, sometimes less deeply stained, occasionally merely lightly tinted.

Epithelial cells were a common feature of the plugs (Figs. 37 to 40). They were of large size, with abundant cytoplasm. The nucleus was sometimes darkly, sometimes lightly stained, or had contracted to form a small darkly stained mass, the chromatin network of which was not well defined. The cytoplasm usually stained more deeply with acid dyes than did that of the epithelial cells lining the tubule, but was less granular in aspect than the latter.

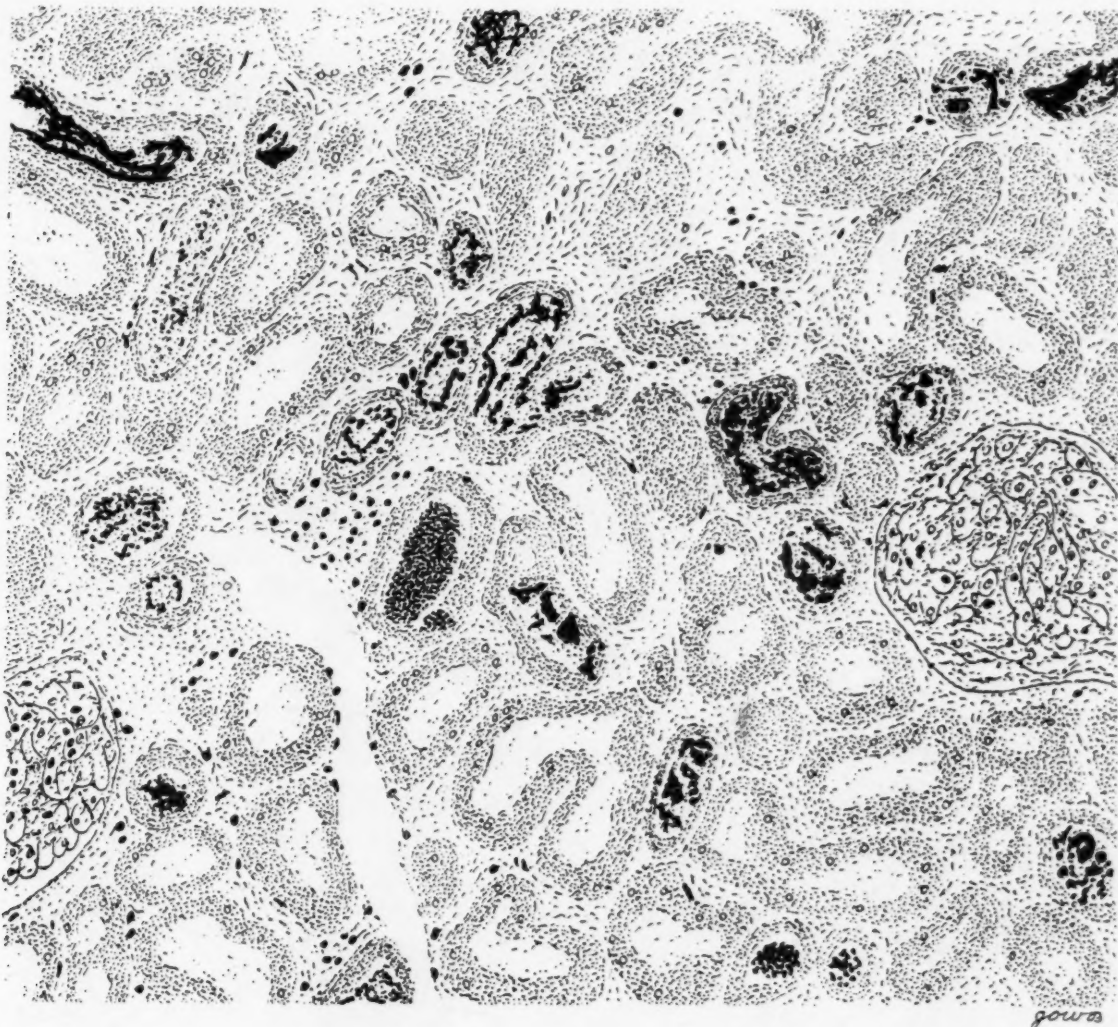
The lightly stained debris sometimes seen in the tubules in addition to the above elements was in part made up of well-defined fine granules,  $2\mu$  to  $5\mu$  or less in diameter, and in part of amorphous flocculent material. Every transition could be observed between the former and the darkly stained granules of smaller size described above.

The most striking feature of the material contained in the lumen of the renal tubules was its multiformity and irregularity, which was much greater than that observed in the renal casts seen in the urine during experimental haemoglobinuria or during blackwater (Figs. 21 to 32).

The uriniferous tubules were considerably distended, the lumen of the convoluted tubules commonly having a diameter of  $40\mu$  to  $50\mu$  (Figs. 33 to 40), and the epithelial lining affected being correspondingly flattened. The same distension affected also the cavity of the Malpighian capsules (Fig. 33), which occasionally contained lightly stained finely granular deposit. Beyond flattening, however, no other abnormal change was recognisable in the epithelial cells of the kidney. The nuclear staining was unaltered, and cloudy swelling was not observed. No brown granules were met with in the epithelial cells of the uriniferous tubules.



In the interstitial tissue of the kidney no cell infiltration or other change was present. The blood vessels of the kidney were usually empty. No malarial pigment was found in the blood vessels or elsewhere. No haemorrhages were seen in the interstitial tissue of



0  $\mu$  150  $\mu$  300  $\mu$

FIG. 41. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Many of the larger renal tubules (mostly convoluted tubules) and some of the smaller contain darkly staining material, partially filling the lumen. This material is in part so darkly stained as to look homogeneous, but where less dense it can be recognised to be made up of irregular granules of varying size. The epithelium of the renal tubules, which in this section is very faintly stained, does not present any unmistakable evidence of degeneration. The interstitial tissue does not exhibit any marked increase in amount, nor any cell infiltration. No malarial pigment can be recognised. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 160$ .

either cortex or medulla, but haemorrhage beneath the capsule of one kidney, already referred to, was observed in Case 7a.

The position and aspects of the renal casts, and the distension of the renal tubules and Malpighian capsules are represented diagrammatically in Fig. 62A.

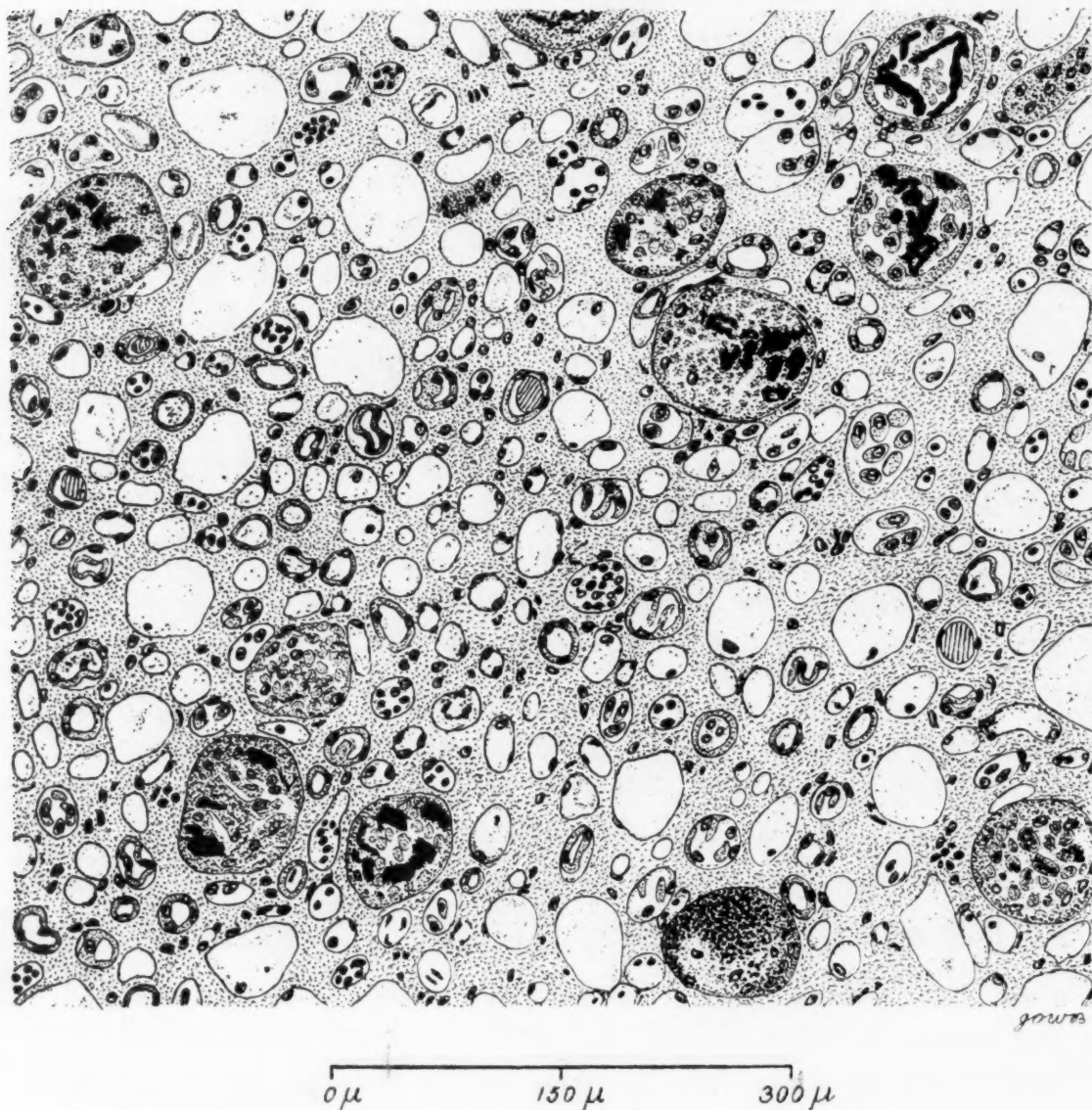


FIG. 42. Transverse section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Many of the collecting tubules are in part occupied by darkly staining material, usually dense in aspect, but in one of the tubules coarse granules are seen. In some of these tubules epithelial cells and debris can also be recognised. Some of the collecting tubules are devoid of epithelial lining and contain a very small amount of flocculent material. Four of the smaller tubules show hyaline casts, indicated by parallel shading. A few blood cells are seen containing red cells darkly stained. The interstitial tissue shows no increase in amount nor is any cell infiltration observable. No malarial pigment can be seen. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 160$ .

We will now refer to the histological changes presented by the kidneys in another attack of blackwater fever (Case 16), in which death occurred seven days after haemoglobinuria had ceased, during which period 640 c.cm. ( $22\frac{1}{2}$  oz.) of urine of specific gravity 1.015 were passed daily (see clinical notes, pp. 235 to 239). Although in this case it

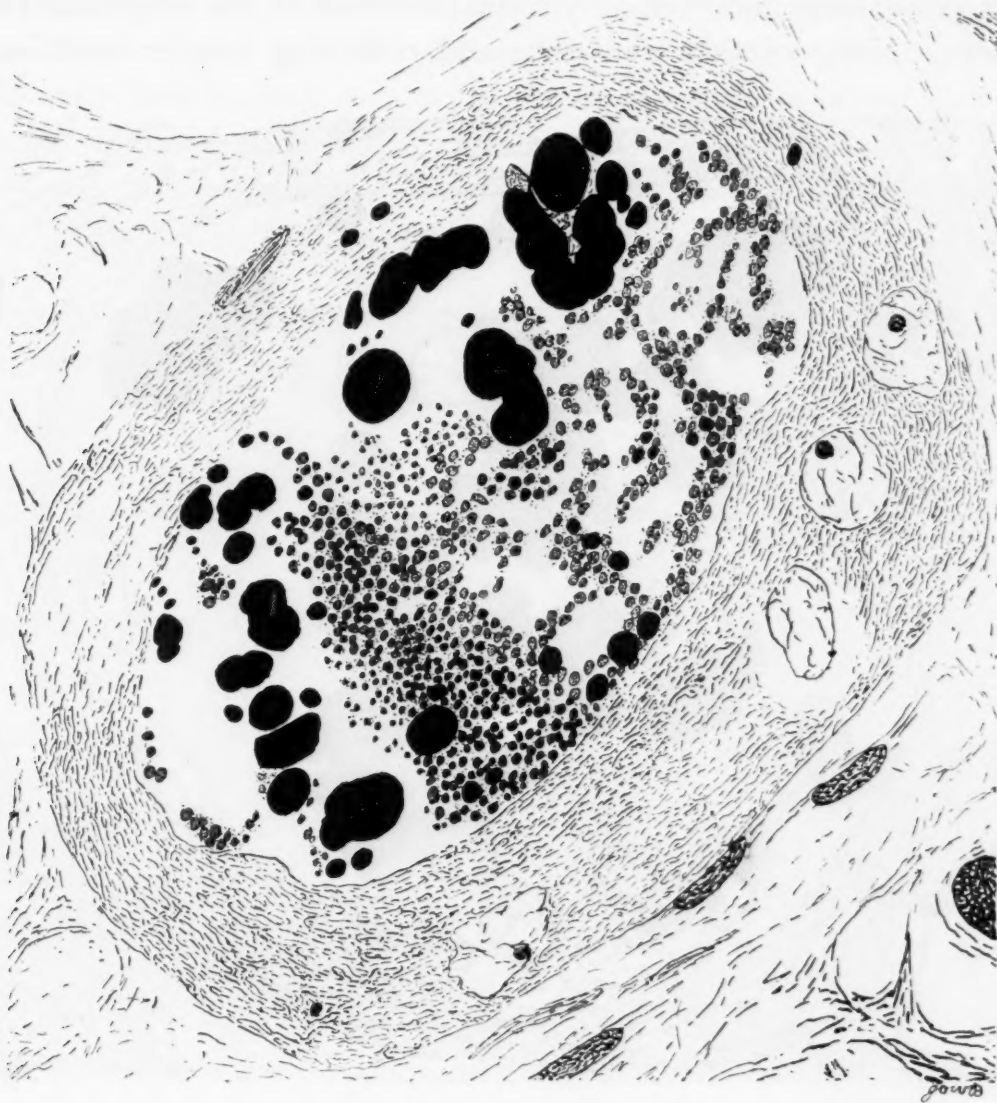


FIG. 43. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Transverse section of renal tubule. The lumen is occupied by darkly stained large coarse granules,  $3\mu$  to  $5\mu$  in diameter, similar to those seen in the preceding Figures, and also by smaller granules, less darkly stained and about  $1.5\mu$  in diameter. The section is lightly stained. Fixed in Flemming's solution. Stained with iron alum haematoxylin  $\times 1400$ .



is not certain that some degree of suppression of urine occurred, the condition of the kidneys offers an interesting comparison with that found in Cases 7a and 11.

In naked eye appearance the kidneys presented little change beyond slight enlargement. Sections of the kidneys (Figs. 41-44 and 57-58) were, however, very similar to those of the preceding two cases. Many of the uriniferous and collecting tubules contained

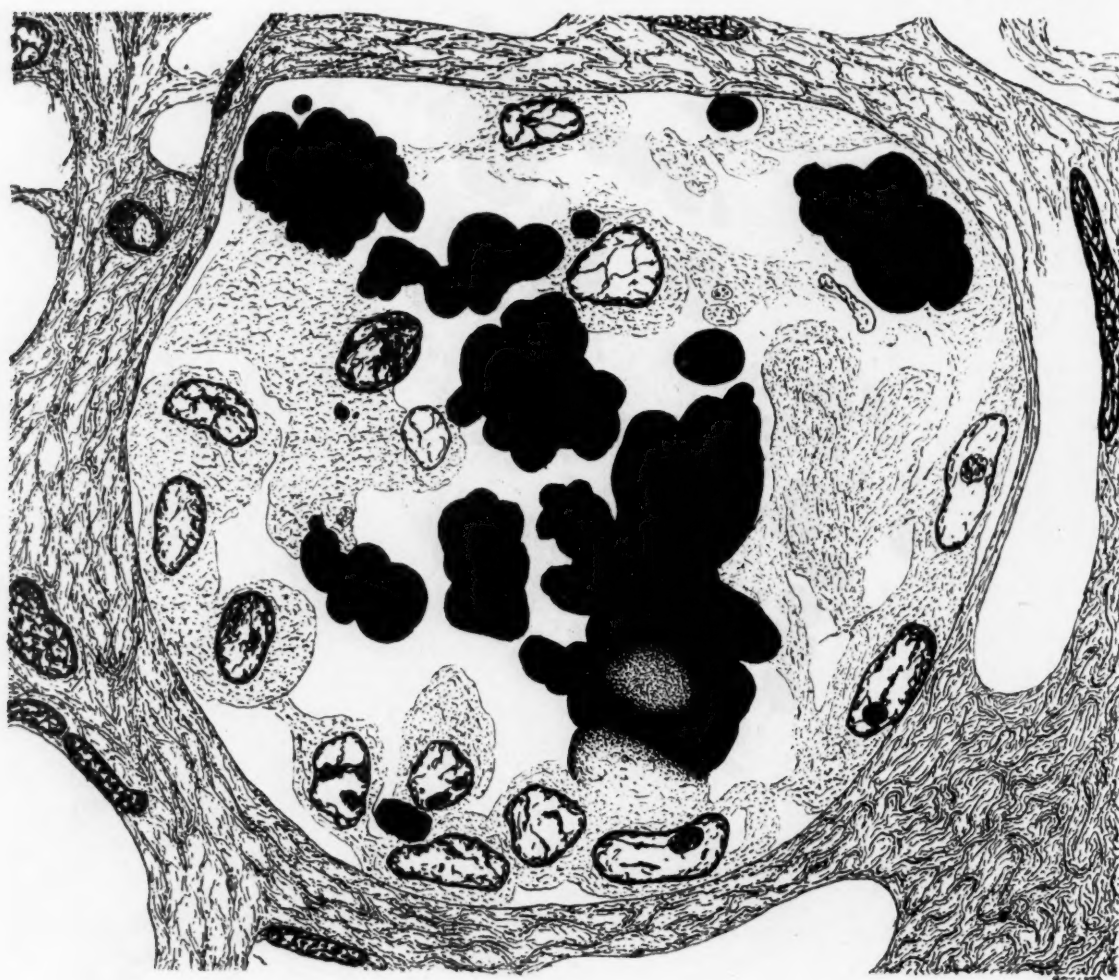
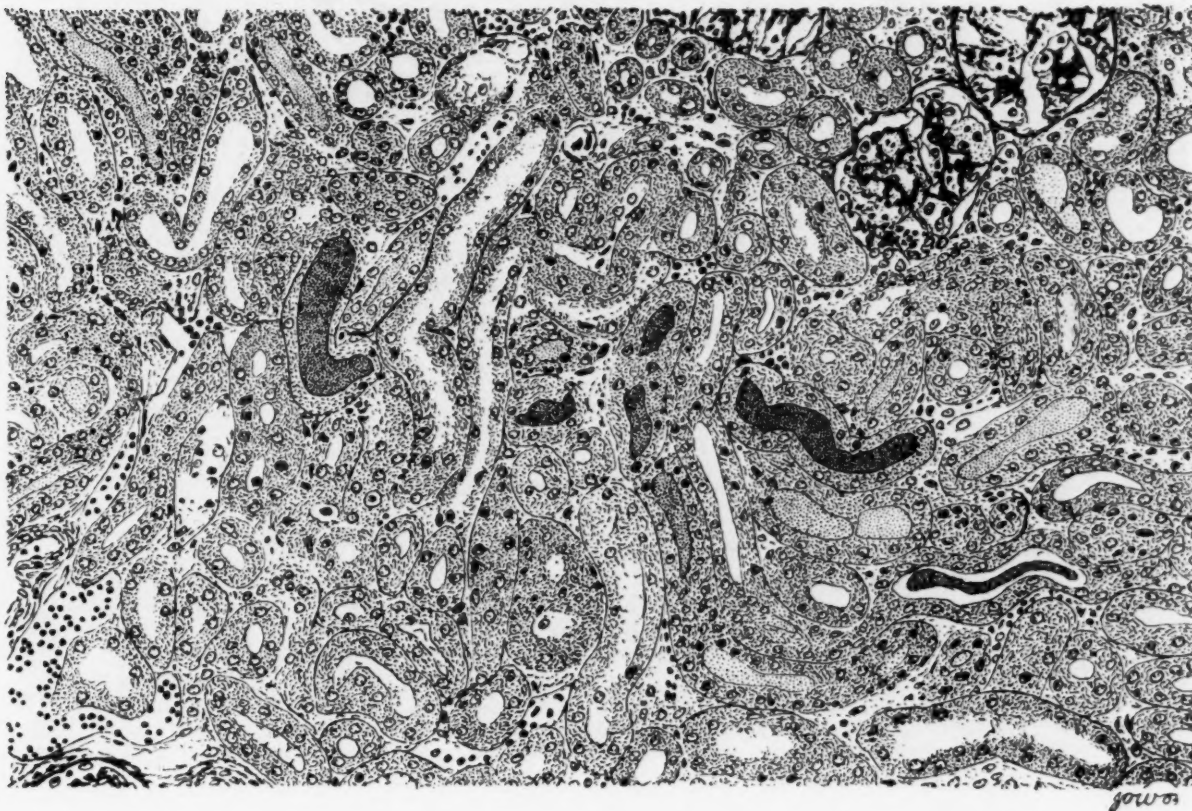


FIG. 44. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A large collecting tubule is shown in transverse section. In the lumen of the tubule are darkly stained coarse granules,  $2\mu$  to about  $5\mu$  in diameter, and masses formed by the coalescence of these granules. Free epithelial cells are also seen within the tubule, among these masses. The cells of the epithelial lining of the tubule are partially detached. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 1300$ .



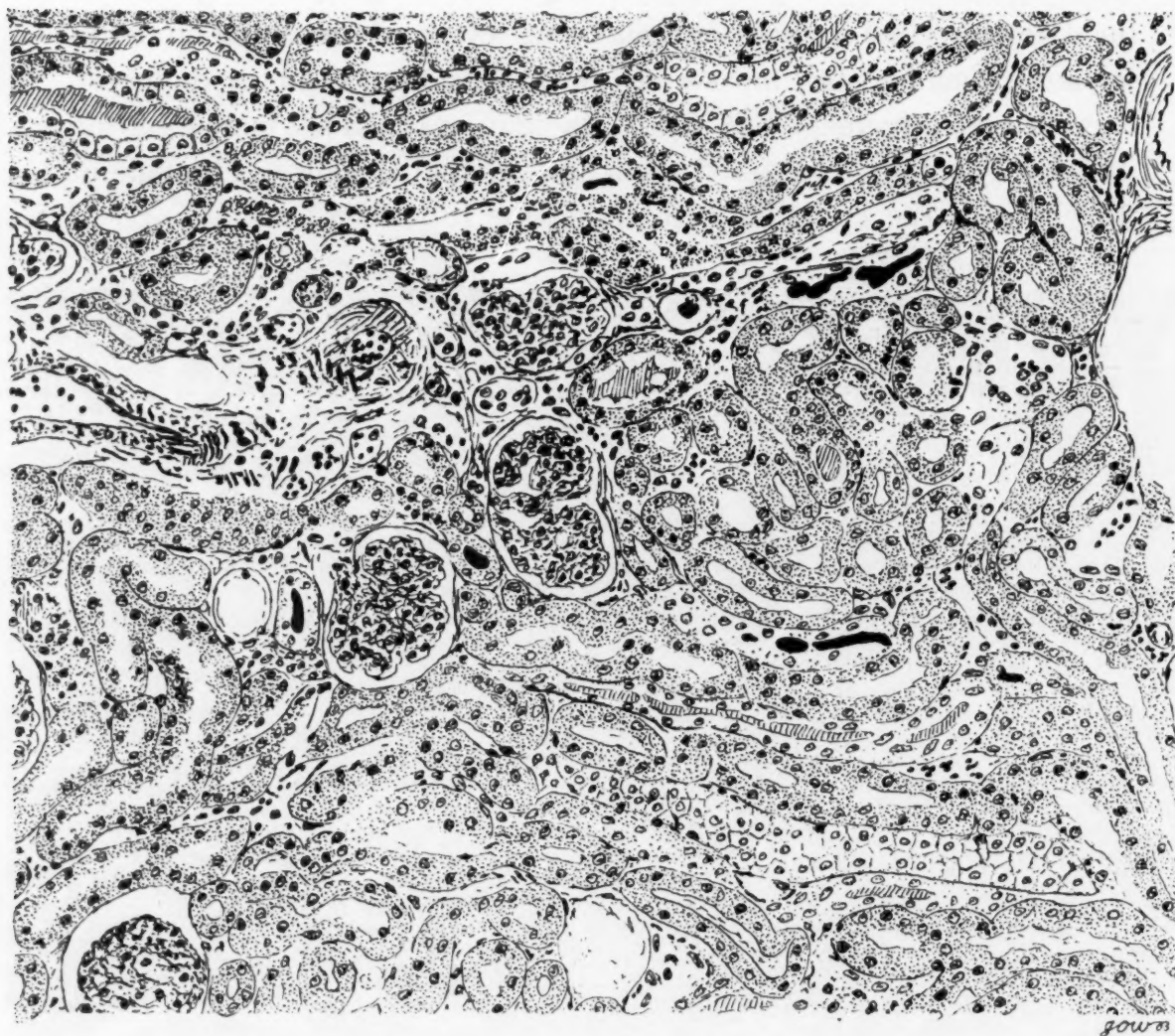
large darkly staining granules and masses composed of groups of granules more or less completely fused together. These granules and granular masses were accompanied by epithelial cells lying free in the lumen of the tubules, and also by a variable amount of lightly staining granular and flocculent material. The individual darkly staining granules were usually of large size, ranging from  $3\mu$  to  $5\mu$  in diameter, and in addition collections of darkly staining granules of much smaller size, measuring  $2\mu$  to less than  $0.5\mu$  in diameter, were frequently met with (Figs. 43 and 57). The lumen of the tubules generally contained numerous epithelial cells. Throughout the kidneys the epithelial cells lining the tubules were more or less



0  $\mu$  150  $\mu$  300  $\mu$

FIG. 45. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In some of the tubules dark masses containing granules are seen. The individual granules are small and vary in number, being generally somewhat scanty. The renal epithelium is healthy and the tubules are not dilated. The interstitial tissue shows no change. Red cells are recognisable in several intertubular capillaries and in one Malpighian capsule. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 170$ .

completely detached (Fig. 44). Some of the tubules, in particular the large collecting tubules, exhibited no epithelial covering, the basement membrane being bare (Fig. 42). Lightly staining material, present in the form of granules,  $1.5\mu$  to  $0.5\mu$  in diameter, and of flocculent deposit, is seen in Figs. 43 and 58. The lumen of the

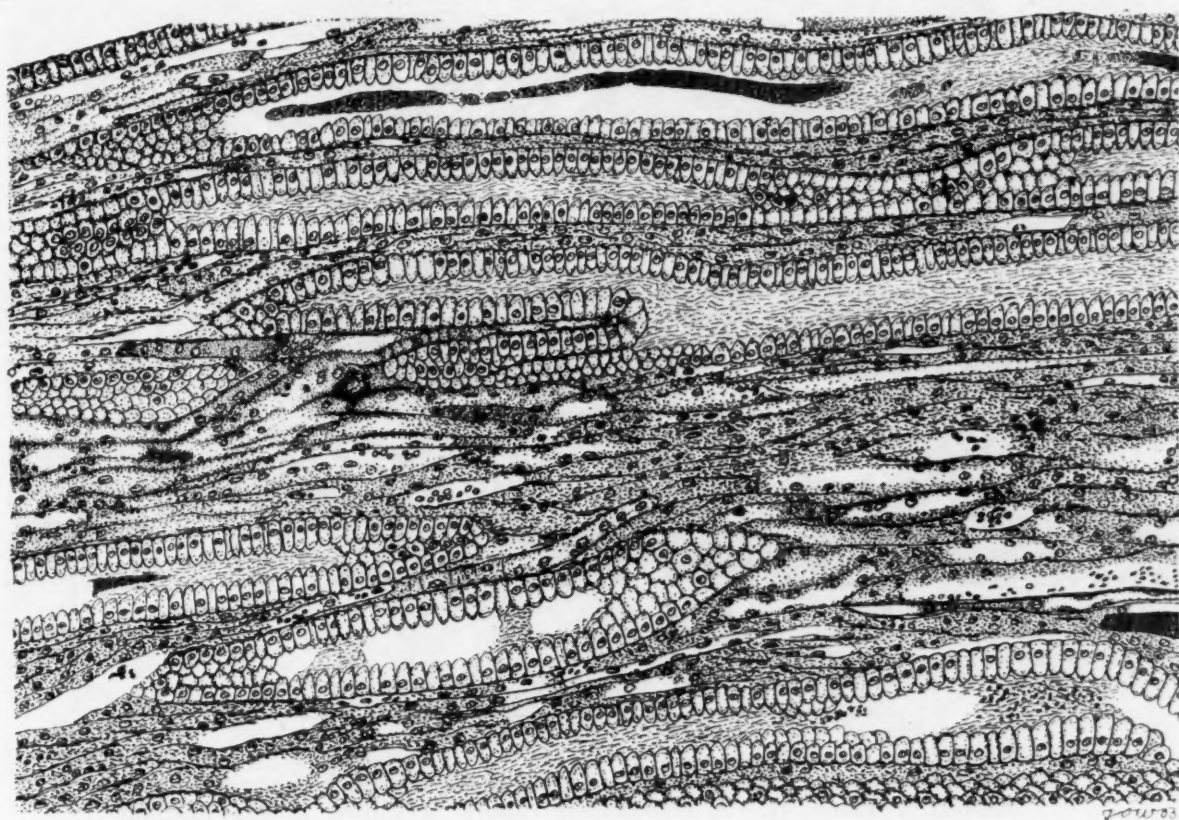


0  $\mu$  150  $\mu$  300  $\mu$

FIG. 46. Another section of cortex of kidney during experimental haemoglobinuria of Rabbit 7, Table 35. The epithelium is healthy in aspect, showing no sign of cloudy swelling or other indication of degeneration. The tubules are not recognisably dilated, but casts of granular material, stained very darkly, are present in six of the tubules. Casts presenting a homogeneous aspect, indicated in the sketch by parallel shading, are seen in five tubules. There is no increase of nuclei or other change in the interstitial substance of the kidney. A few of the blood capillaries contain red cells, stained black. No malarial pigment is seen. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 155$ .



tubules was wide, but there was not the marked distension with flattening and thinning of the epithelial lining seen in the two preceding cases. The interstitial tissue showed no cell infiltration, nor was any recognisable increase of connective tissue present. No evidence of renal haemorrhage was found, and the blood vessels were free from malarial pigment. It will thus be seen that the chief points of difference, as regards the condition of the kidneys, between this case (16) and the preceding cases (7a and 11), in which suppression of urine occurred, are: the absence of distension of the renal tubules in degree sufficient to cause flattening and thinning of



0μ 150μ 300μ

FIG. 47. Longitudinal section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In four of the large collecting tubules granular casts are seen partly occupying the lumen of these tubules. The granules are fine, but vary somewhat in size and also in number in different situations. In addition some slightly staining flocculent material is observable in the tubules. The renal epithelium is healthy and the tubules are not dilated. No change is present in the interstitial tissue. A few of the blood capillaries contain red cells darkly stained. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 170$ .

the epithelial lining; the frequency with which small granules,  $2\mu$  to less than  $0.5\mu$  in diameter, were met with; and the much greater extent to which the renal epithelial cells were loosened and detached.

The condition of the kidneys of the rabbit in experimental haemoglobinuria was also studied. In illustration of the changes observed, we give sketches of sections made: during haemoglobinuria (eighty-five minutes after the injection of dissolved haemoglobin, Experiment 7, Table 35), Figs. 45-49 and 59-60; twenty-two hours after injection and fourteen hours after cessation of haemoglobinuria (Experiment 9, Table 35), Figs. 50-51.

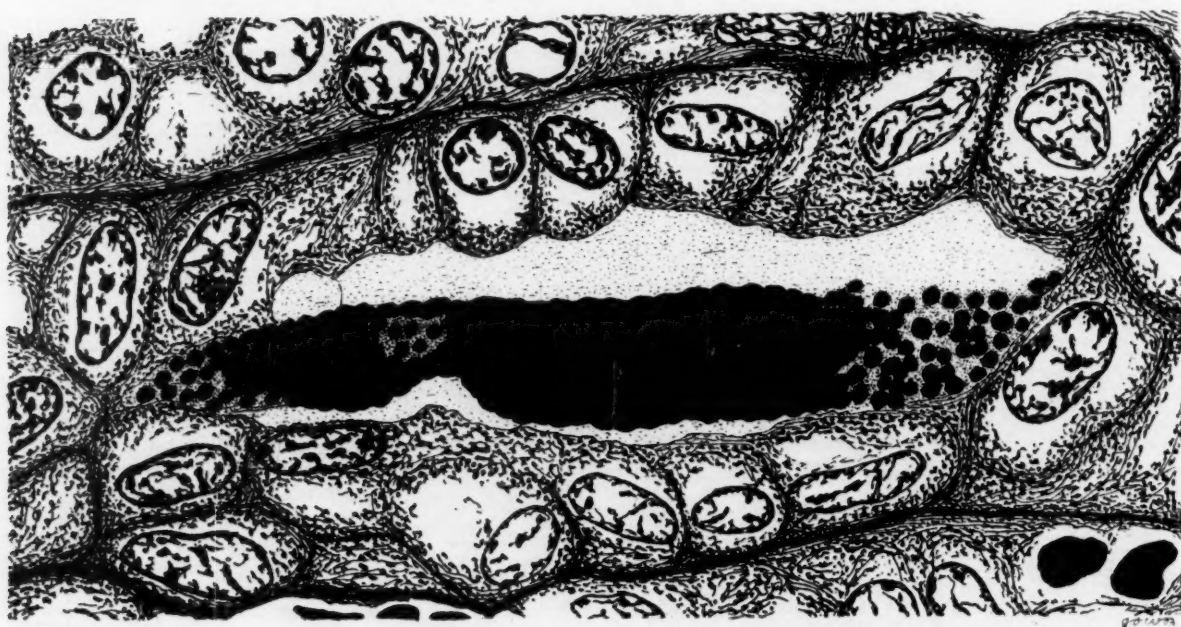


FIG. 48. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A convoluted tubule is shown in longitudinal section, containing a granular cast, which does not completely fill the lumen of the tubule, the intervening space being occupied by hyaline material in which the cast is embedded. At each extremity of the cast, and near its centre, the individual granules become distinct and have a diameter of  $1\mu$  to  $2\mu$ ; elsewhere they are packed together so densely that their individual outlines become unrecognisable. The cytoplasm of the renal tubules presents a finely granular aspect. Below two entire red blood cells and part of three others, stained black, are shown. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 1250$ .

In each case the naked eye aspect of the kidneys presented no obvious change from the normal, the colour of the kidney being unaltered, the pyramid natural in appearance, and no brown stippling



being recognisable. On cutting sections of the first kidney (Experiment 7, Table 35), the only change observable was the occasional presence, in some of the renal tubules, of granular material having the aspect of the casts already described in the urine (Figs. 21-24). This appeared to be scattered here and there in all parts of the uriniferous and straight tubules, but was absent from the Malpighian capsules. The granules varied in size from  $2\mu$  to less than  $0.5\mu$ ;

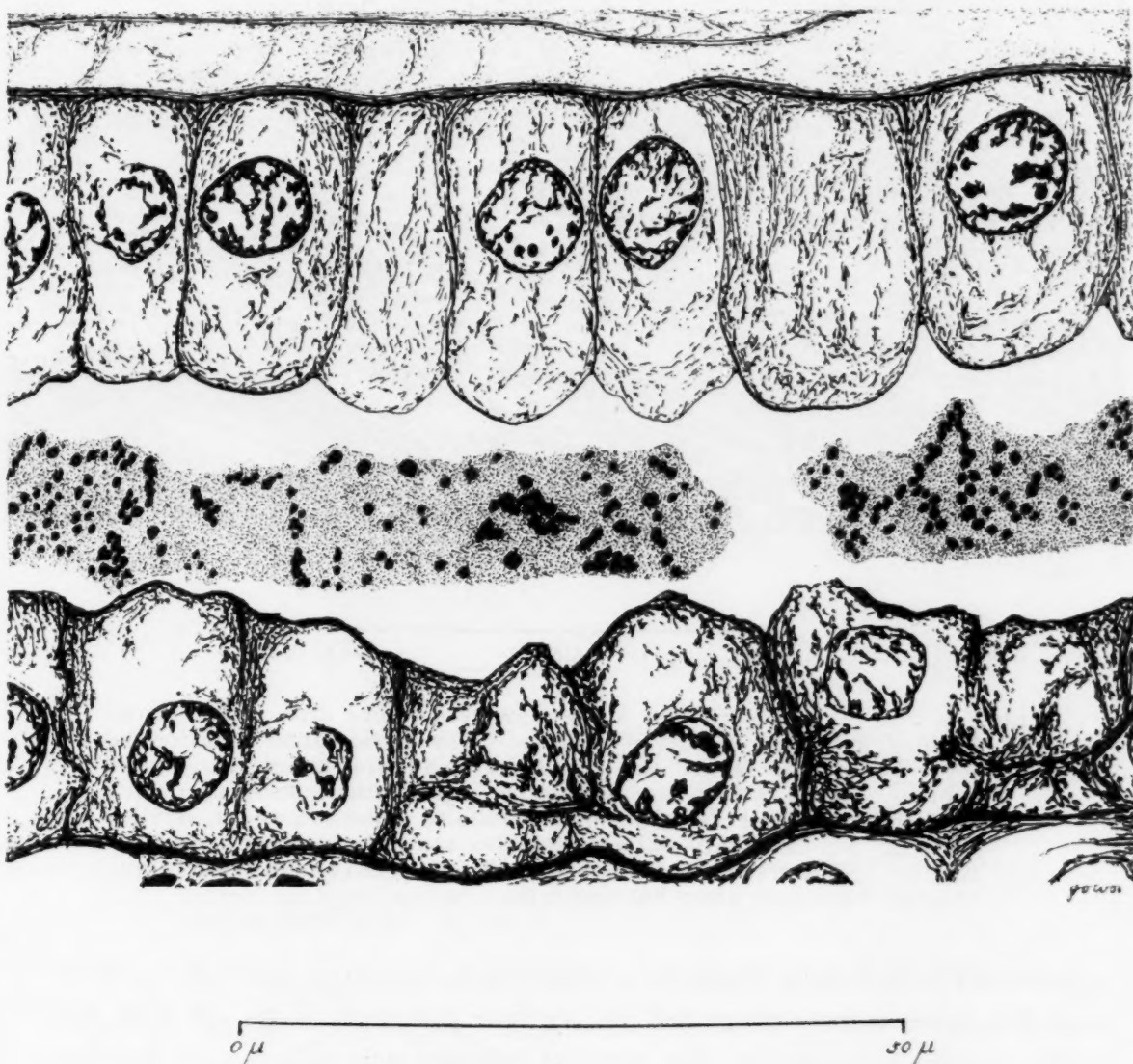
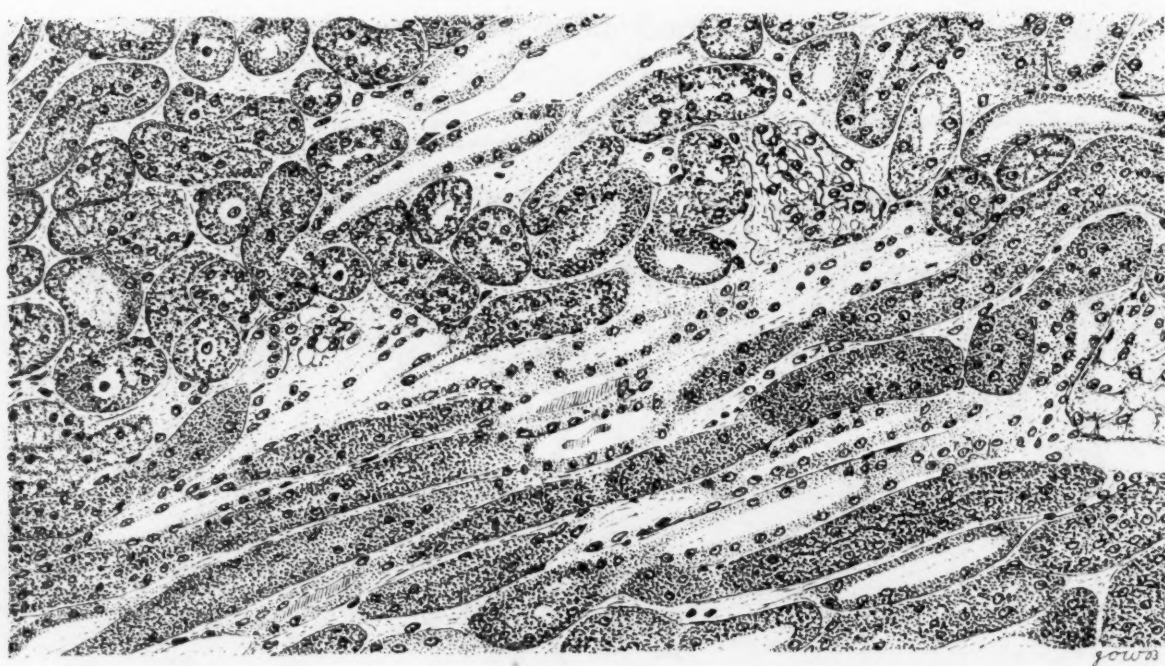


FIG. 49. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In longitudinal section a collecting tubule is shown, partly occupied by a cast consisting of semi-opaque flocculent material containing here and there darkly stained granules  $0.5\mu$  to  $1\mu$  in diameter. The granules are mostly discrete; in some places they are in contact, but they do not appear to have undergone fusion. The renal epithelium is healthy in aspect. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 1400$ .

they were brown in colour, in the unstained condition, and did not appear to increase in size as the pelvis of the kidney was approached. They stained readily, but were more easily decolourised than the larger granules seen in the kidneys in Cases 7a and 11. The casts varied in denseness, and the hyaline material which formed their



0  $\mu$  150  $\mu$  300  $\mu$

FIG. 50. Section of cortex of kidney fourteen hours after experimental haemoglobinuria. Rabbit 9, Table 35. The section shows portions of three Malpighian bodies and a number of tubules, chiefly convoluted tubules, all of which are normal in aspect, the epithelium being unaltered. Three of the smaller tubules show casts of hyaline material, but no darkly-staining granules are to be seen in any of the tubules. None of the tubules are dilated. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 155$ .

basis contained sometimes few, sometimes numerous granules. Most of the casts were surrounded by hyaline material (Figs. 48 and 49). The lumen of some of the cortical tubules was filled with hyaline material which resembled haemoglobin, inasmuch as it readily took up acid dyes (Figs. 45 and 46). No change was seen in the blood vessels. The renal epithelium was perfectly normal in aspect.

The condition of the kidneys during experimental haemoglobinuria is represented diagrammatically in Figs. 61B and 62B.

Similar renal plugs were observed in cattle and dogs during red-water due to piroplasmosis (Fig. 52).

The kidneys of the rabbit fourteen hours after the cessation of haemoglobinuria were, however, in all respects normal, no granular material being present in the tubules (Figs. 50 and 51).

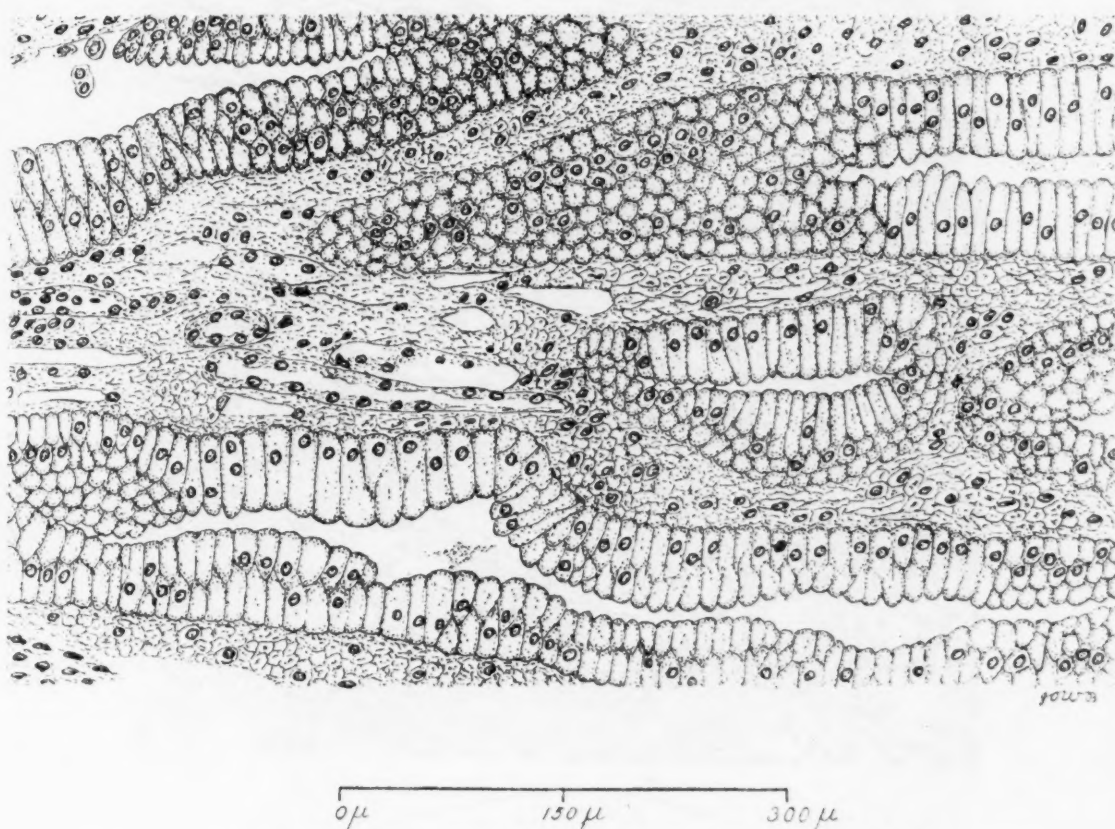
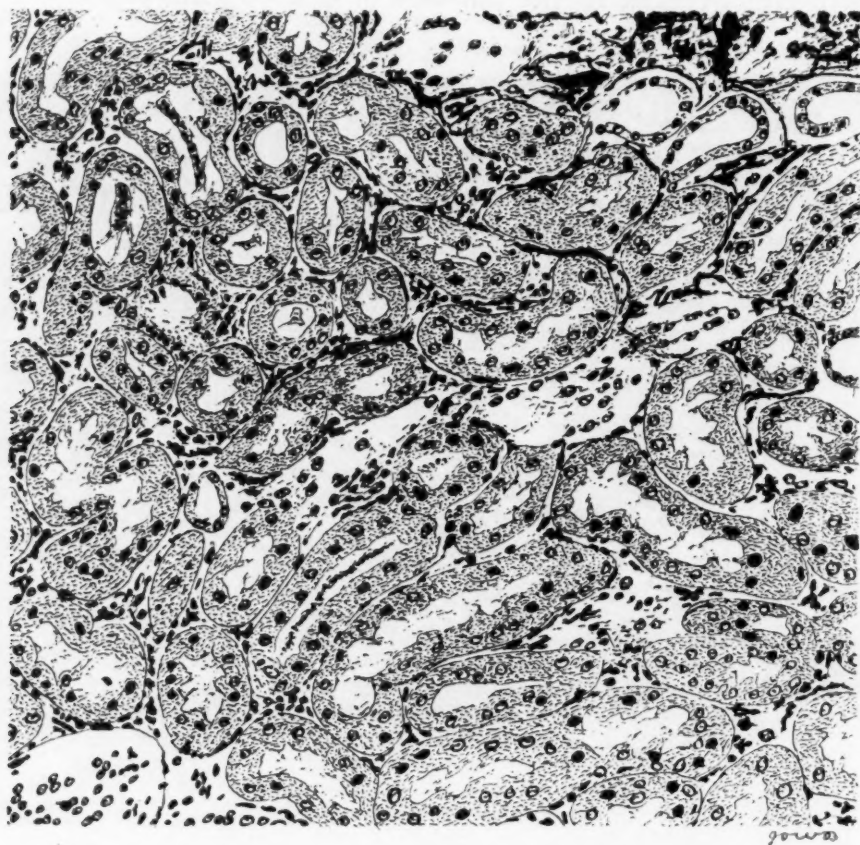


FIG. 51. Longitudinal section of medulla of kidney fourteen hours after experimental haemoglobinuria. Rabbit 9, Table 35. Three collecting tubules are seen, also several small tubules. The epithelium is everywhere healthy in aspect. A small amount of deposit is seen in the lumen of some of the tubules, but no darkly-staining material. There is no dilatation of the tubules, and the interstitial tissue is normal in aspect. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 155$ .

In the experiments upon rabbits, already described (Tables 35, 37), in which haemoglobinuria was produced by the intravenous injection of dissolved haemoglobin, an increased flow of urine occurred. Up to the present we have not obtained suppression of urine after injection. It will be, however, of interest to refer at this point to a group of experiments carried out by different observers, in which haemoglobinuria has been followed by anuria. In these experiments haemoglobinuria was produced by the injection of a



haemolytic serum (Ponfick,\* Joannoviez†) of a solution of potassium chlorate (Marchand,‡ Lebedeff§), of glycerin (Lebedeff,§ Afanassiew||), of iodine (Lebedeff,§ Afanassiew||), of pyrogallie acid (Afanassiew||), and of toluylendiamin (Afanassiew||). By all these agents



0  $\mu$  150  $\mu$  300  $\mu$

FIG. 52. Section of cortex of kidney of bull during redwater due to piroplasmosis. In three of the convoluted tubules casts with coarse irregular granules, darkly stained, are seen partly filling the lumen of the tubules. The epithelium is not degenerated and the tubules are not dilated. No alteration of the interstitial tissue is recognisable. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 170$ .

\*Experimentelle Beiträge zur Lehre von der Transfusion, Virchow's Arch., 1875, B. 62, S. 273.

†Experimentelle Untersuchungen über Ikterus, Zeitschr. f. Heilkunde, 1904, B. 25, S. 25.

‡Über die Intoxication durch chlorsaure Salze, Virch. Arch., 1879, Bd. 77, S. 455.

§Zur Kenntniss der feineren Veränderungen der Nieren bei Hämoglobinausscheidung, Virch. Arch., 1883, B. 91, S. 267.

||Über die pathologisch-anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Ikterus verbundenen Vergiftungen, Virch. Arch., 1884, B. 98, S. 460.



haemoglobinuria may be obtained and sometimes anuria also. In the latter case the kidneys and spleen have been found enlarged, and the former has shown dark medullary striation and brown cortical stippling, while the uriniferous tubules have contained plugs of dark brown granular material, the condition being similar to that already

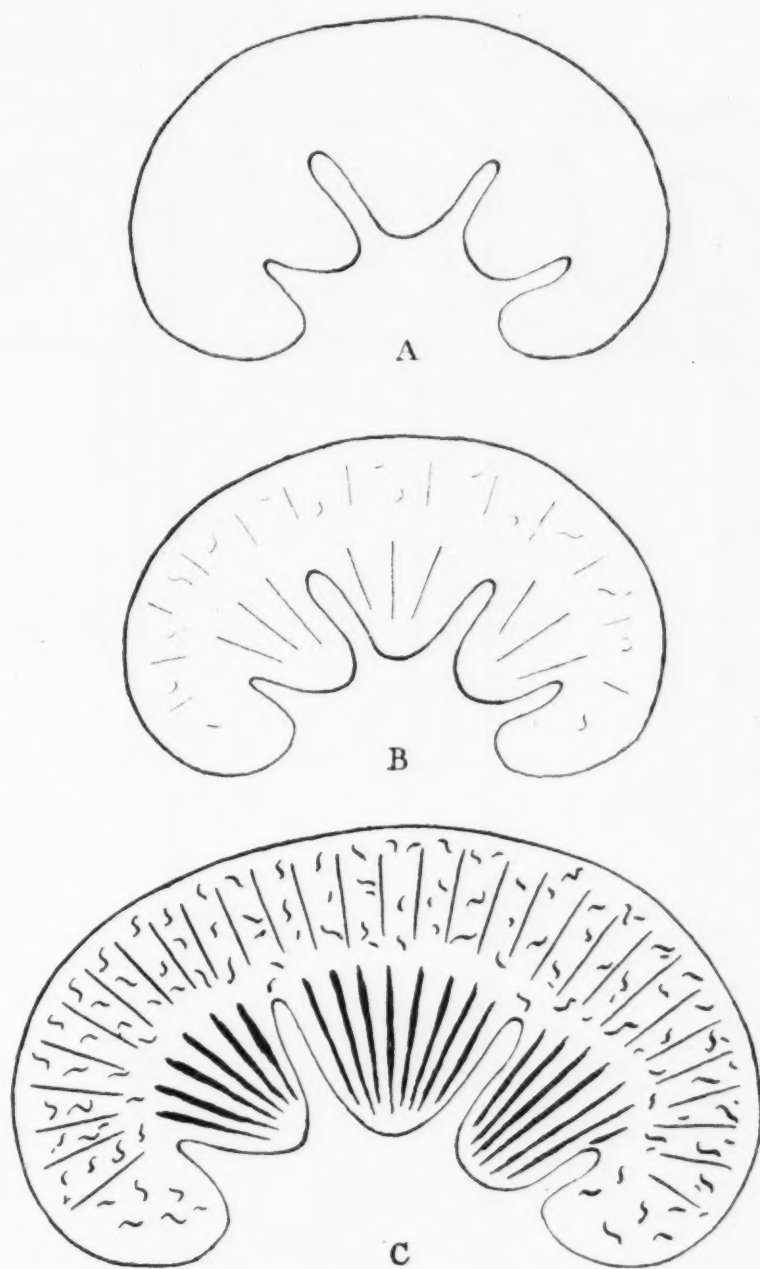


FIG. 61. Diagrammatic representation of the condition of the kidney in health (A), during experimental haemoglobinuria of rabbit (B), and in suppression of urine following upon blackwater fever (C). In B small casts are present in some of the uriniferous and collecting tubules; in C large casts are present in many of the uriniferous and collecting tubules. In the latter considerable enlargement of the kidney is also observed. For the sake of comparison B is represented as tri-papillary instead of being uni-papillary, as in the rabbit.

described as occurring in suppression of urine in blackwater fever. The condition of the animals, however, differed in several important particulars from that obtaining in blackwater fever, for, in addition to the profound toxic effect produced by the agents employed, marked changes in the appearance of the red blood cells such as have not

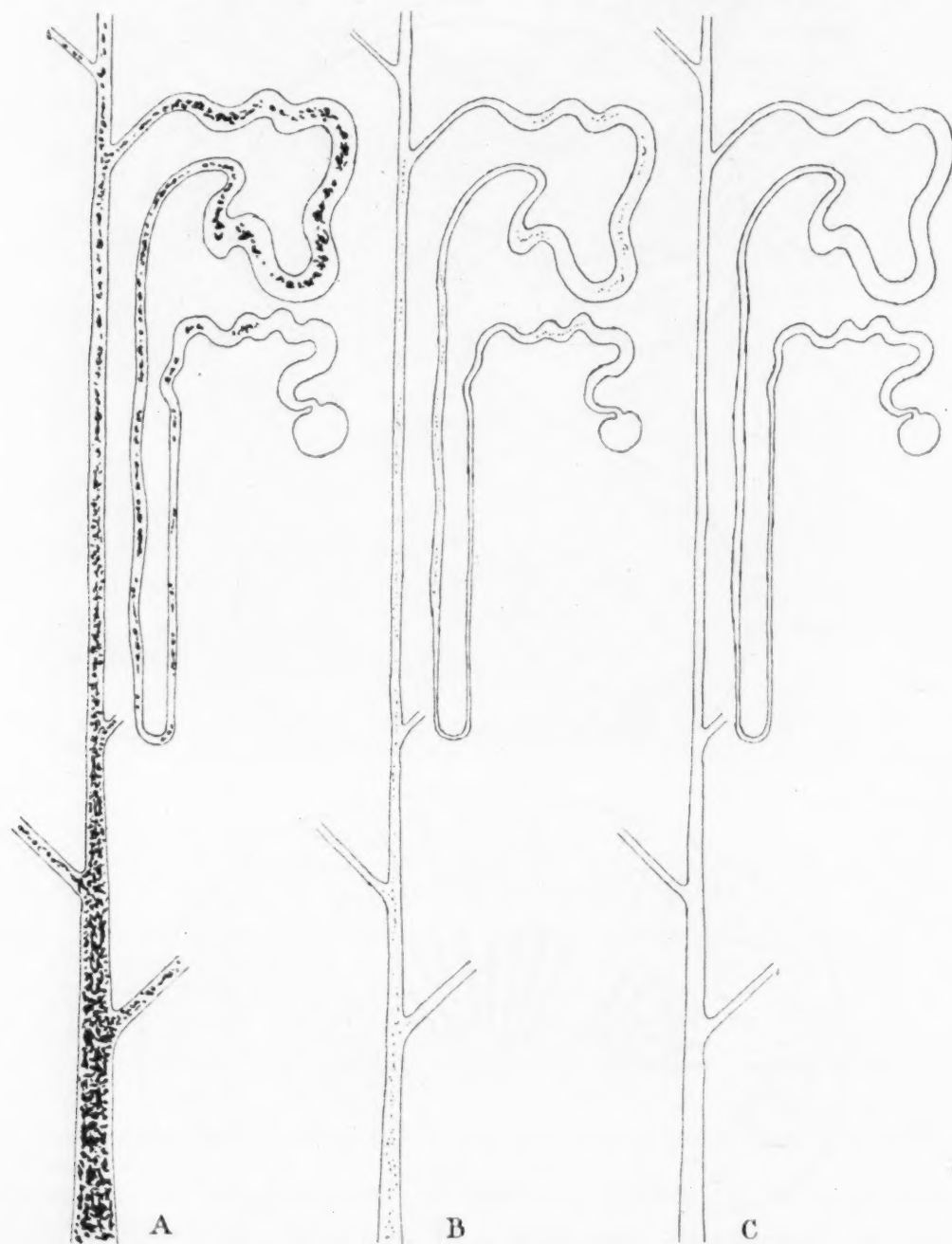
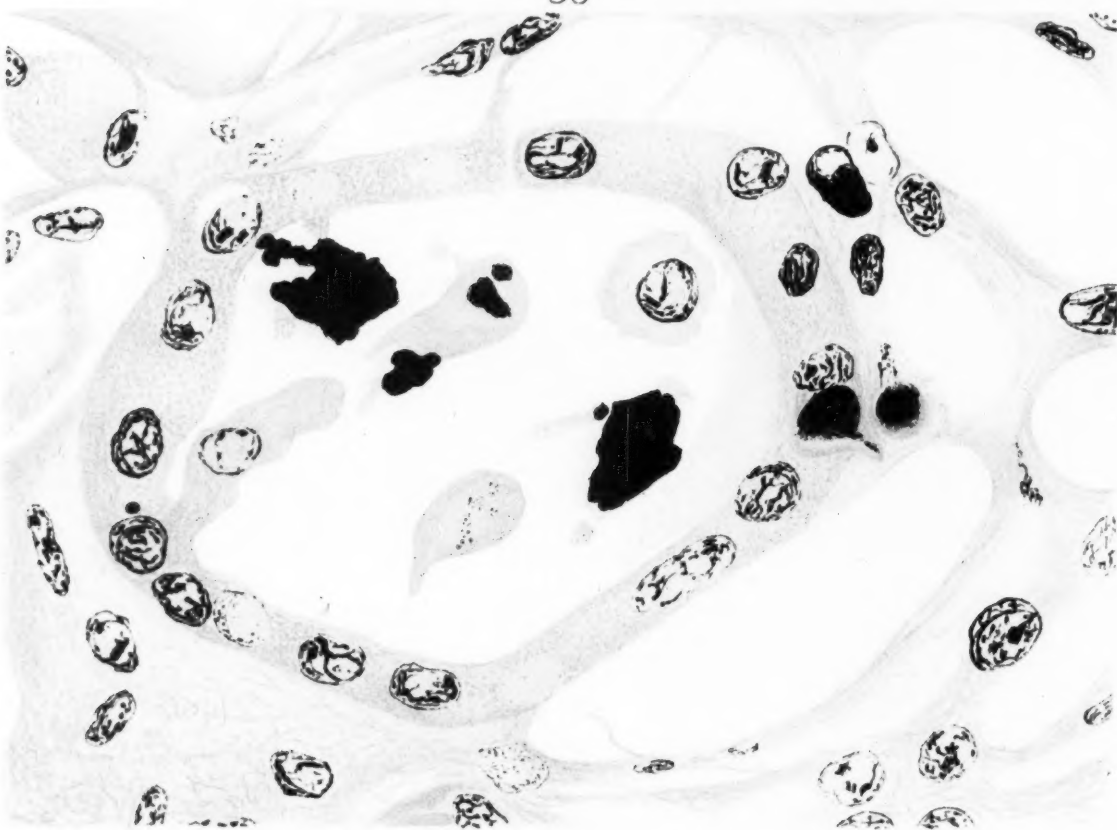


FIG. 62. Diagrammatic representation of the condition of the glomerular capsule, uriniferous tubule and collecting tubule in health (C), during experimental haemoglobinuria of rabbit (B), and in suppression of urine following upon blackwater fever (A). In B fine granules, in A coarse granules and masses are present in the renal tubules, which in A are also dilated.

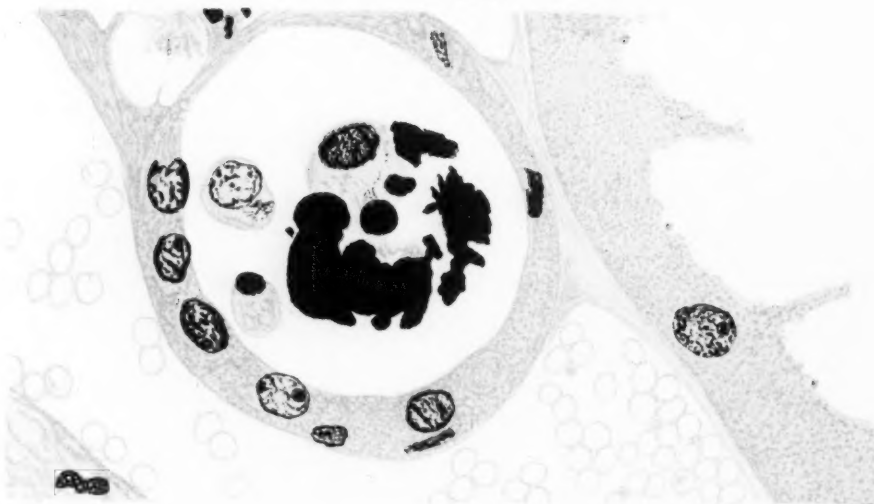
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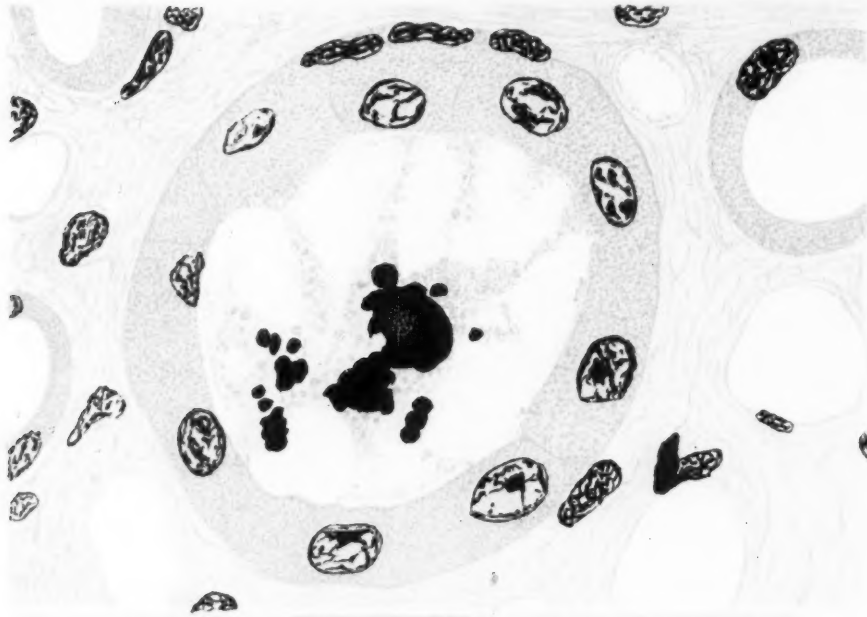
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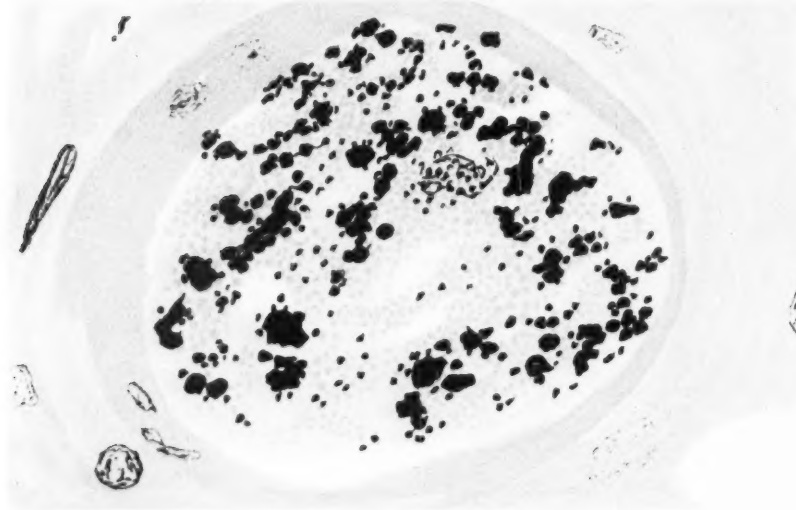
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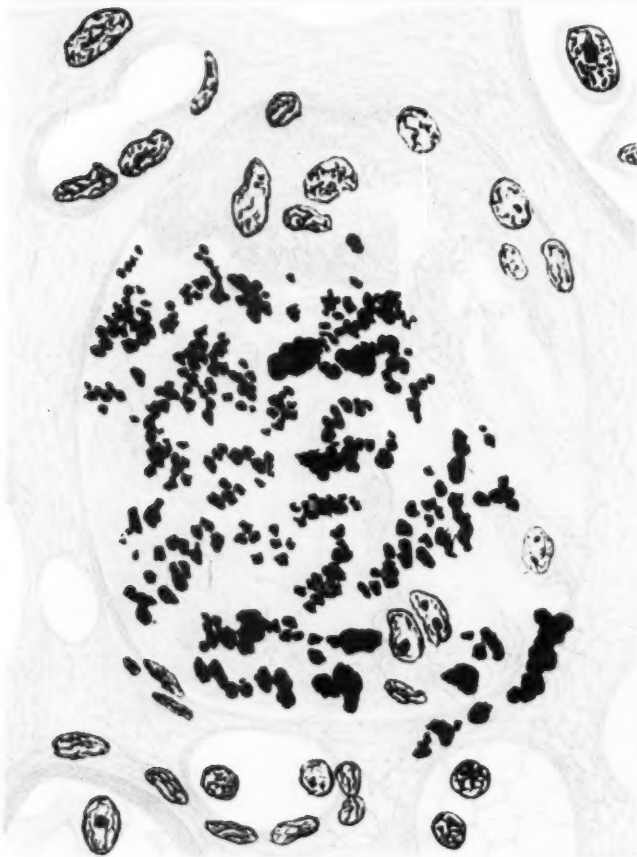
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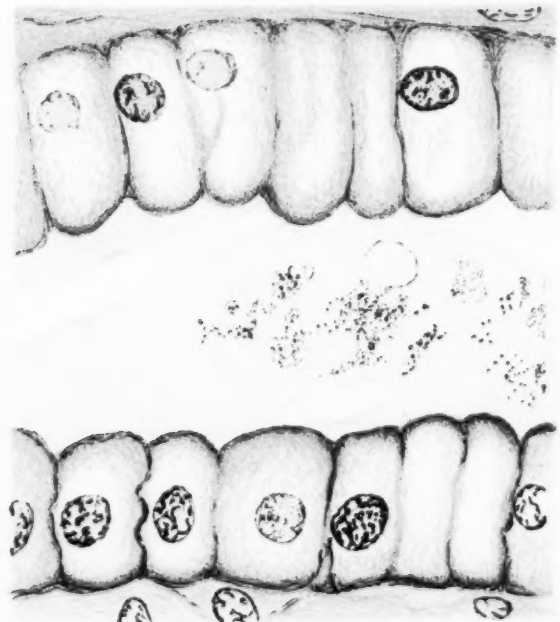
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been observed in blackwater fever, in particular fragmentation of the red cells, were present, and in the kidneys epithelial degeneration was frequently observed. Unfortunately, in consequence of the complexity of these experiments, in which, to use a mathematical expression, several variables occur together, it is difficult to be quite sure of the relation of the pathological conditions present to the haemoglobinuria and anuria, or to be certain of the identity of the anuria so produced with that occurring in blackwater fever, in spite of the similarity of the aspect of the kidneys in the two cases. To ascertain the mechanism of production of the haemoglobinuria and anuria in such investigations it is essential to experiment as far as possible with a single pathological condition at a time. The experiments in question, however, if not as illuminating as could be desired, are very suggestive and of considerable interest.

From the foregoing description of the condition of the kidneys in suppression of urine in blackwater fever, the mechanism of this process becomes at once quite clear. The suppression is not a functional state, but is a purely mechanical process due to blocking of the collecting tubules by plugs composed of densely packed dark reddish granules, identical with the casts observed in the urine during suppression (Cases 7a and 11, Fig. 32), and passing by every transition into the casts ordinarily found during blackwater not attended with suppression, the difference between the two lying in the large size of the granules and greater firmness of the casts in the former as compared with the latter, and also in the firm adhesion to the epithelial lining of the tubules in the former case.

The effect of the plugging is seen in the distension of the uriniferous tubules and Malpighian capsules leading to the considerable general enlargement of the kidney observed at death (Figs. 61 and 62).

#### DESCRIPTION OF ILLUSTRATIONS

FIG. 53. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A convoluted tubule is shown across obliquely. The lumen of the tubule, which is dilated, contains numerous darkly stained masses measuring  $7\mu$  to  $25\mu$  across, consisting of granules,  $2\mu$  to  $5\mu$  in diameter exhibiting varying degrees of coalescence. The renal epithelium is not markedly changed. No free epithelial cells are seen in the lumen of the tubule. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .

- FIG. 54. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A collecting tubule is shown in transverse section, in the lumen of which are seen darkly stained, two large irregular masses, and several smaller collections of granules. The individual granules measure from  $2\mu$  to  $4\mu$  in diameter. Four free epithelial cells are also present in the lumen of the tubule. The cytoplasm of the latter is more homogeneous in aspect than that of the epithelial wall of the tubule, which is unchanged in appearance. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 55. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. An ascending limb of Henle's loop is seen in transverse section. The lumen of the tubule contains darkly stained masses, composed of partially coalesced granules, the smallest of which are about  $2\mu$  in diameter. In addition three free renal cells are seen in the tubule. To the right is a portion of a convoluted tubule, and below and to the left a blood vessel. The renal epithelium is very granular in aspect. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 56. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. A large collecting tubule is shown in transverse section, containing darkly stained granules  $1\mu$  to  $3\mu$  in diameter, arranged in groups of varying size, and accompanied by lightly stained flocculent and finely granular material. The epithelial wall of the tubule is unaltered in aspect, as is also the interstitial tissue. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 57. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A renal tubule, probably an ascending limb of Henle's loop, is shown cut across transversely. The wall of the tubule, which retains its epithelial lining, is faintly stained so that its outlines are indistinct. In the lumen of the tubule are numerous darkly stained granules, varying in size from less than  $1\mu$  to about  $4\mu$  in diameter, in part arranged in groups. In addition a large amount of lightly tinted granular material is also recognisable. Fixed in Flemming's solution. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 58. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A collecting tubule, lightly stained, is seen in transverse section. The lumen of the tubule is filled with darkly stained granules, mostly arranged in groups. The individual granules measure  $1\mu$  to  $3\mu$  in diameter. The epithelial lining of the tubule is partially detached. Unlike the preceding section, there is very little unstained debris contained within the lumen of the tubule. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 59. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A renal tubule is shown, containing granules  $0.5\mu$  to  $1\mu$  in diameter, which occupy a relatively small portion of the lumen of the tubule. These granules are darkly stained, but slightly less so than is represented in the Figure. No other debris is present in the tubule. The epithelial lining of the tubule wall is well defined, some of the divisions between the individual epithelial cells being recognisable. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .
- FIG. 60. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A collecting tubule is shown cut across longitudinally. The lumen of the tubule is for the most part occupied by lightly staining flocculent material containing a few very fine darkly stained granules the diameter of the largest of which does not exceed  $0.5\mu$ . The epithelial lining of the tubule is normal in aspect. Hardened in alcohol. Stained with iron alum haematoxylin.  $\times 850$ .



The enquiry, which has some bearing upon the prophylaxis of suppression, presents itself; is it necessary, in order that this plugging may occur, that the flow of urine should be diminished? Obviously if the flow were very rapid the tendency to accumulation of granular material in the tubules would be diminished, and with it also the tendency to blocking. The question turns largely upon whether the granular material which is present becomes denser through the absorption of water by the epithelium of the tubules. If this occurs the formation of dense plugs is evidently partly conditioned by the relation subsisting between the rate of flow of glomerular fluid on the one hand and the rate of removal of water from the lumen of the tubules on the other hand. If the latter is sufficiently rapid no retardation of the former may be required in order that plugging may take place.

In view of the mechanical blocking of the kidneys in suppression of urine in blackwater fever, it is astonishing that this process should still be attributed to a purely functional disturbance, a nervous reflex or vasomotor inhibition of secretion of urine, a view which is now chiefly of historical interest. By A. Plehn\* a nervous inhibition of glomerular secretion is regarded as the primary and essential factor in this condition.

Another point of interest in connection with suppression of urine in blackwater fever is the enquiry whether, when once suppression has been established, the plugs may yet be dislodged and the flow of urine re-established. As a matter of fact such cases occur: four cases are referred to by Werner,† in which after two or more days anuria secretion of urine was re-established. In Cases 7a and 11 (pp. 195 and 217) it has already been mentioned that dislodged plugs were constantly

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\* Ätiologie und Pathogenese des Schwarzwasserfiebers, Virch. Arch., 1903, B. 174, S. 509. On page 519: "das plötzliche Stocken der Urinsekretion stellt aufangs vielleicht ebenfalls einen rein functioneller Vorgang dar, und es folgt erst sekundär die mechanische Verlegung der Harnkanälchen durch Coagulieren der Eiweissüberladenen Flüssigkeit." J. de Haan, Die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- und Tropenhygiene, 1905, B. 9, S. 22, explains suppression as in part due to mechanical obstruction by renal plugs, in part due to low glomerular pressure in consequence of which the pressure of the urine is insufficient to drive out the plugs (pp. 30-31). Our observations on experimental haemoglobinuria do not afford any evidence of the occurrence of clotting in the tubules; on the contrary the constituents of the blood which lead to clotting do not pass through the kidney, haemoglobin alone being eliminated.

†Loc. cit., p. 14.

present in the scanty urine passed during the period of suppression. We may, therefore, surmise that temporary, and it may be also partial, plugging is in reality a not unfrequent occurrence in blackwater fever and produces for a time a partial or even complete arrest of the flow of urine from a few or from the majority of the renal tubules, such obstruction afterwards passing away, the plugs becoming dislodged by the pressure of urine behind. This condition may be suspected whenever the granular casts found in the urine are large and very firm. It is well known that the amount of urine passed when haemoglobinuria makes its appearance may be scanty. This is illustrated by the following cases, coming under our observation, in which the quantity of urine secreted during the first twenty-four hours of haemoglobinuria was 248 c.cm. in Case 2; 344 c.cm. in Case 3; 170 c.cm. in Case 6a (next day 85 c.cm.); 530 c.cm. in Case 10; and 800 c.cm. in Case 15. So marked a reduction as occurs in the first four cases can only be attributed to renal blocking.

The fluid passing into the bladder during suppression obviously consists in part of urine. This is shown by the circumstance that the suppression exhibits in different cases varying degrees of incompleteness, indicating that the blocking of the renal passages is correspondingly incomplete, as is further illustrated by the occasional re-establishment of the flow of urine after suppression of several days' duration. It does not, however, follow that the whole of the fluid passing into the bladder during suppression is urine. The coagulable proteid contained in the urine, which was considerable in amount in Cases 7a and 11, varying from  $\frac{1}{4}$  column to  $\frac{2}{3}$  column, may enter the renal passages as a result of damage to the renal epithelium consequent upon plugging of the collecting tubules, but a certain amount probably comes also from portions of the tubules in which plugs carrying renal epithelium (Figs. 27, 31 and 32) have become detached and have been discharged into the renal pelvis, so that the basement membrane of the tubules has become exposed. In such cases the lymph contained in the tissue spaces of the kidney would drain into the tubules and mix with the urine. Our investigation, unfortunately, came to an end just as the study of this problem was commenced. It is desirable that further information should be obtained as to the nature of the proteids present, as well as the amount of urea and other nitrogenous substances in the urine during suppression.

**SUMMARY**

1. During experimental haemoglobinuria dependent upon haemoglobinaemia, as also in the haemoglobinuria of blackwater fever, granular casts of varying size, sometimes very soft, sometimes firm and dense, often containing degenerated nuclei derived from the epithelium of the renal tubules, were met with in the urine (Figs. 21-31).

2. During suppression in blackwater fever (Cases 7a and 11) the urine contained very large firm casts with exceedingly coarse granules, often surrounded externally by epithelium derived from the ducts of Bertini, in which these casts were formed (Fig. 32).

3. The amber-coloured urine passed during suppression contained a large amount of coagulable proteid. The average daily amount of urine passed during nine days of suppression was in one case (7a) 28 c.cm., in another case (11) 66 c.cm.

4. Suppression of urine in blackwater fever is of mechanical origin, due to a blocking of the renal tubules.

# V. THE MECHANISM OF PRODUCTION OF BLACKWATER.

The condition of the urine and blood plasma in respect of dissolved haemoglobin in blackwater fever has been studied in Section 3. In the same section also, the relation between haemoglobinaemia and haemoglobinuria has been determined experimentally in rabbits, while the condition of the urine and of the kidneys in suppression of urine in blackwater fever has been described in Section 4. The problem now remaining to be considered is whether the mechanism of production of blackwater is identical with the process induced experimentally in rabbits. Before the consideration of this point can be entered upon it will be necessary to make further reference to the condition of the haemoglobin in the urine in blackwater fever, and in particular to the presence of red cells in the urine, to allude again to the changes undergone by haemoglobin in the urine, and to describe the methods adopted for the estimation in urine of haemoglobin, both when unchanged and when altered by the action of urine.

TABLE 43. Characters of the urine in blackwater fever, in respect of brown coloration, unchanged haemoglobin in solution, and red blood cells.

No. of Case	Colour	Haemoglobin in solution	Red blood cells	Estimation of haemoglobin (maximum amount)
1	Porter coloured	Present	Present	—
2	Chocolate coloured	Absent	"	—
3	Porter coloured	Present	"	1.4 % (Fig. 10)
4	Dark red	—	—	—
5	Claret red	Present	—	—
6	Porter coloured	—	Present	—
6a	Brown	Absent	"	0.8 %
7	Porter coloured	"	Absent	0.2 %
7a	"	Present	Absent at first, appeared afterwards	1.2 % (Fig. 11)
8	"	"	Present	0.2 % (Fig. 12)
9	"	—	—	—
10	"	Present	Absent at first, appeared afterwards	2.6 % (Fig. 13)
11	"	"	"	2.6 % (Fig. 14)
12	Dark red	"	Absent	2.2 % (Fig. 15)
13	(Red)	—	—	—
14	Porter coloured	Present	Absent	3.0 % (Fig. 16)
14a	"	"	"	1.8 % (Fig. 17)
15	"	"	"	3.8 % (Fig. 18)
16	"	"	"	0.8 % (Fig. 19)
17	"	"	Present	3.5 % (Fig. 20)



In Table 43 the condition of the urine in respect of haemoglobin is given in all except three (4, 9, 13) of the cases of blackwater fever coming under our observation. It will be seen (Column 3) that in thirteen out of sixteen cases haemoglobin was present in solution, in the form of oxyhaemoglobin, in amounts varying from 0.2 per cent. to 3.8 per cent., while in addition a further amount of haemoglobin was changed into the brown substance, exhibiting no bands in the solar spectrum, already referred to (p. 51), which gives rise to the porter-colour of the urine. The total amount of dissolved haemoglobin passing into the urine consists of these two fractions, which are determined by the methods given below. Although in three cases (2, 6a, 7) haemoglobin in solution could not be recognised by spectroscopic examination of the brown or porter-coloured urine passed, nevertheless dissolved haemoglobin was in all probability originally present, but had been broken up by the action of the urine (cp. Table 25, p. 53); in Case 2 the urine was retained for several hours before being voided, and micro-organisms were present in the bladder (see clinical notes, p. 178); in Case 6a the percentage of haemoglobin in the urine was 0.8 per cent.; and in Case 7 the percentage of haemoglobin present was also very small, and the urine had stood for some time before being examined. In a subsequent attack (7a) the last patient passed urine containing dissolved haemoglobin.

When the urinary deposit obtained after centrifugalisation was examined (Column 4, Table 43), red blood cells were found to be present in ten out of sixteen attacks, but in three of these (7a, 10, 11) red cells were at first absent from the urine and made their appearance subsequently (clinical notes, pp. 195, 207 and 217). As a rule the red cells were not present in large numbers. They were in fact usually much less numerous than the casts, but an exception occurred in Case 2, in which they were present in very considerable numbers, so as to form at the bottom of the urine glass a brownish red deposit visible to the naked eye. In this case there were a number of decolourised red cells and also red cell stromata. The red cells were sometimes crenated, but beyond this and the partial decolouration just referred to they did not present any other abnormal appearance.

In order to estimate haemoglobin in urine, the following three methods were employed; the first two being employed for the estimation of unaltered dissolved haemoglobin, and the third for the

estimation of both unaltered haemoglobin and also haemoglobin which had been broken up by the action of the urine.

*Method 1.* The urine was centrifugalised and the supernatant liquid diluted with a measured volume of distilled water until the percentage of haemoglobin as judged by the naked eye was about 0.15 per cent.\* The diluted urine was then put into the half cell of von Fleischl's haemoglobinometer. If the dilution was found unsuitable a fresh dilution was made until a concentration was obtained which could be easily matched. The haemoglobinometer scale was previously standardised by means of solutions containing known percentages by volume of healthy human red cells; this having been done, the percentage of haemoglobin in the diluted urine could be at once determined from the scale numbers.

In many cases no difficulty was found in making a determination of haemoglobin by this method. Difficulty arose, however, when the haemoglobin had been largely converted into the brown substance already referred to, a change which is likely to occur when the urine has been retained in the bladder for a long time before being passed, or after being voided, has been allowed to stand for some time before examination. This change is of course relatively more marked in urines containing small, than in urines containing large, percentages of haemoglobin. Another source of difficulty in matching the colour of the diluted urine with that of the tinted glass of the haemoglobinometer occurs when dissolved haemoglobin is discharged in small amount into urine which is itself of a dark brownish amber colour, as not unfrequently happens in malarial patients. Matching also becomes difficult when the urine in the bladder contains bacteria, as occurred in Case 2, the turbidity thus caused not being completely removable by centrifugalisation. When from any of these causes it becomes impossible to employ the above described method for the estimation of dissolved haemoglobin, recourse may be had to the next method.

*Method 2.* The urine was diluted, if necessary, with a known volume of distilled water until, in a column one to two centimetres

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\* This rough comparison may be made by putting the centrifugalised urine into a test-tube, filling another test-tube with a 0.15 per cent. solution of haemoglobin and comparing the two, the former being further diluted or a lesser dilution prepared, as may be necessary. With a little practice, however, it becomes possible to obtain the required dilution with sufficient approximation by the naked eye without comparison.

high; the oxyhaemoglobin bands lying between the solar lines D and b were separate and of suitable depth of shade. By the aid of a haemocrit a solution of haemoglobin, containing 0.2 per cent. by volume of healthy human red cells, was next prepared. A glass cell one or two centimetres high was then filled with this solution, while the diluted urine was placed in a second cell, the height of which could be varied. The haemoglobin bands of the second cell were then, by varying the height of the column employed, made to match those of the first, for which purpose a Zeiss comparison spectroscope was employed.

By this method it is possible to estimate percentages of haemoglobin in cases in which colouring matter is present in the urine in amount sufficient to render haemoglobinometer determination difficult or impossible. This method may, of course, be used when the preceding method is also available. The results obtained by the two methods correspond closely. The first method is more rapidly carried out, and is, therefore, when available, preferable to the second.

*Method 3.* The estimation was made by boiling the solution of haemoglobin. Ten c.cm. of the centrifugalised urine, which did not as a rule require filtering, were rendered fairly acid with acetic acid, boiled and after cooling transferred to a graduated centrifugal tube. Centrifugalisation was continued until the bulk of the precipitate became constant. Its volume was then measured. Previously by means of the haemocrit, a number of specimens of urine (with the addition of 5 per cent. to 25 per cent. of water) were prepared containing known percentages, by volume, of healthy red blood cells (in the moist condition), which were subsequently laked. To these solutions an equal volume of urine was added, and the solutions rendered very slightly acid with acetic acid and then boiled. The bulk of the centrifugalised precipitates was next determined as above described, and from this a table constructed, which permitted the precipitates obtained in haemoglobinuria to be expressed in percentage form. As this method depends upon the precipitation of globulin, and is not affected to any considerable extent by the change undergone by haemoglobin in the urine, it may therefore be employed to determine with fair approximation the total amount of haemoglobin originally present in the urine. The method is, however, only approximate, since the volume of the precipitate varies



somewhat with different specimens of urine, and is also affected by the rate of centrifugalisation employed. Table 44 gives the average bulk of precipitate obtained with varying percentages of dissolved haemoglobin in slightly diluted urine. By graduating the centrifugal tube employed in percentages of wet red cells, instead of in cubic centimetres, the former could be read off directly and all calculation avoided. It was found that when an equivalent amount of oxalated blood plasma was added (the red cells require to be separated from the blood plasma for convenience in laking) so that (dissolved) haemoglobin and blood plasma were present in their natural relation, the bulk of precipitate obtained was increased by 20 per cent. to 25 per cent.

TABLE 44. Bulk of precipitate obtained on boiling 10 c.cm. of slightly acid urine containing varying percentages of dissolved haemoglobin.

Percentage of haemoglobin contained in urine of sp. gr. 1.014 diluted with 5% to 25% of water.	Bulk (after centrifugalisation) of precipitate obtained upon boiling after previous slight acidification with acetic acid.
6.5%	3.00 c.cm.
5.0%	2.28 c.cm.
4.0%	1.82 c.cm.
3.0%	1.36 c.cm.
2.0%	0.91 c.cm.
1.0%	0.46 c.cm.
0.5%	0.23 c.cm.
0.25%	0.11 c.cm.

By one or the other of these methods the percentage and total amounts of haemoglobin given in Tables 43 and 45 were determined.

In ten attacks of blackwater fever the total amount of haemoglobin passed into the urine was estimated (Table 45) and was found to be represented by between about 1.5 gm. (Case 7) and 75 gm. (Case 14) of wet red cells. In the cases given in Table 45 it will be observed that there is a general correspondence between the percentage and the total amount of haemoglobin, the higher the former the higher the latter. This relation, in the light of the experiments made upon rabbits, given in Table 35, would appear to be merely a coincidence. It will be noted that in the two cases in which suppression of urine occurred (7a and 11) the total amount of haemoglobin appearing in the urine, equivalent to 9 g. of wet red



cells in Case 7a and 22 g. in Case 11, was not so high as in Cases 14 and 17, not of unusual severity, in which the total amounts of haemoglobin were equivalent to 75 g. and 32 g. of wet red cells respectively.

TABLE 45. Amount of haemoglobin passing into the urine in blackwater fever.

No. of Case.	Haemoglobin present in urine during attack, expressed in terms of equivalent weight of wet red cells.
3	12 g.
7	1.5 g.
7 a	9 g.
10	8 g.
11	22 g.
14	75 g.
14 a	20 g.
15	19 g.
16	6.5 g.
17	27 g.

The presence of red cells in the urine in blackwater fever suggests the inquiry whether the haemoglobinuria of blackwater fever may not be really dependent upon renal haemorrhage. In the earlier descriptions of cases of blackwater fever the condition of the urine was often described by using the term haematuria, the condition of haemoglobinuria not having at that time become differentiated from that of haematuria. Subsequent writers recognised that the condition present in blackwater fever was haemoglobinuria, and, so far as we know, no recent writer has described haematuria as a sequence of the administration of quinine in malarial subjects. In the haemoglobinuria produced by the administration of potassium chlorate or other poisonous substances, or by the injection of a haemolytic serum (see p. 128), the presence of red blood cells in the urine, in addition to dissolved haemoglobin, is recorded, but in our experiments on rabbits this did not occur. The possibility of blackwater being primarily due to renal haemorrhage was impressed upon us in particular by Case 2 (pp. 178 to 181), in which the red cells were very numerous, while the urine, after centrifugalisation, although porter-coloured, did not exhibit oxyhaemoglobin bands on spectroscopic examination. If renal haemorrhage really occurred in blackwater fever, the red blood cells escaping into the urinary passages would necessarily become laked in all cases in which the specific gravity of

the urine was 1·004 or less, for in urine of or below this specific gravity laking occurs almost immediately when red cells are added. If the urine is of the specific gravity 1·006 to 1·008, laking occurs with more or less slowness, and in urine whose specific gravity is 1·010 or upwards, laking does not occur to any considerable extent. If renal haemorrhage were the cause of the haemoglobinuria of blackwater fever the site of the haemorrhage would necessarily be the glomeruli of the kidneys, for only here could the blood readily find its way into the urine, while on the other hand marked interstitial haemorrhage would be ordinarily readily discoverable post-mortem, which is not the case. Furthermore, if renal haemorrhage were the cause of the haemoglobinuria of blackwater fever, then the haemoglobinaemia observable in this condition would be a secondary consequence of the renal haemorrhage, and would be explicable only on the assumption that some of the dissolved haemoglobin was taken up by the renal epithelium and passed on into the blood stream.

On the other hand, the experiments made upon rabbits (Table 35), in which the effect of simple haemoglobinaemia, produced with the animal's own haemoglobin, was studied, taken in conjunction with the circumstance that haemoglobinaemia is present during the haemoglobinuria of blackwater fever, suggests very strongly that in this affection the primary condition is haemoglobinaemia.

It remains to be considered, therefore, how far these two alternative hypotheses, namely, primary glomerular haemorrhage and primary haemoglobinaemia, are capable of accounting for the phenomena of blackwater fever, and, if possible, to decide which of the two is concerned in giving origin to blackwater.

The extent to which the theory of a primary haemoglobinaemia is in harmony with the various facts already ascertained respecting blackwater fever will be first considered, and then the remaining hypothesis will be criticised in the same manner. Owing to the importance of this enquiry, it will be dealt with at some length.

1. From the experiments made on rabbits (Table 35) it is evident that a primary haemoglobinaemia reaching to as much as 0·95 per cent. of haemoglobin, such as occurs in blackwater fever, would be attended with the appearance of haemoglobin in the urine. Our results do not enable us to say how large the maximum percentage of haemoglobin in the blood plasma in blackwater fever

may be. It is probable, judging from the rapidity with which haemoglobin disappears from the circulating blood in the rabbit, that our observations, which were of necessity few in number, represent less than the maximum percentage. Nor are our observations sufficiently numerous to enable us to state the duration of the haemoglobin, but the observations available in Table 33 and Figs. 13, 18 and 20, suggest that in blackwater fever the discharge of haemoglobin into the blood plasma is a slow process, and the duration of the haemoglobinaemia is therefore much longer than in the experimental haemoglobinaemia of Table 35. The amount of haemoglobin passing into solution in the blood plasma cannot be determined. It would not be possible to make even an approximate calculation of this amount in blackwater fever until the curve representing the degree of haemoglobinuria from beginning to end of the blackwater had been ascertained and the rate at which disappearance of haemoglobin from the living body took place determined. It is, however, easily possible to obtain a figure representing the minimum amount of haemoglobin passing into the blood stream. For example, in an individual whose blood measured 4500 c.cm. and contained 45 per cent. of red cells and 55 per cent. of plasma, if the blood plasma were found on examination during blackwater fever to contain as much haemoglobin as would be present in 1 per cent. of its volume of red blood cells in the moist condition, then the total amount of haemoglobin discharged into the blood plasma would be not less than that contained in  $2475 \times 0.01 = 24.75$  c.cm. of the patient's red cells, or 45 c.cm. of his blood prior to the onset of haemoglobinaemia. The amount actually passing into solution in his blood plasma would be greater than this, for, on the one hand, a single observation of his blood plasma would not reveal the maximum percentage of haemoglobin, and, on the other hand, the whole of the haemoglobin would not be discharged into the blood plasma in the course of a few minutes, as in the experiments recorded in Table 35. Since in the observations recorded in Table 50 the duration of individual attacks of haemoglobinuria ranged from four hours or less to four days, the amount of blood destruction would appear to represent a considerable portion of the total amount of blood present, and would be sufficient to account both for the watery condition of the blood generally observed when the finger is pricked,



and also for the marked anaemia which usually attends blackwater fever.

2. The hypothesis of a primary haemoglobinaemia\* is in harmony with another characteristic phenomenon of blackwater fever, namely, the appearance of granular casts in the urine. The experimental work on rabbits shows that haemoglobinaemia is necessarily attended with the formation of these casts. The exact mode in which the granular material is formed remains in both cases undetermined. The experiments made by different observers as to the site of elimination of haemoglobin are still regarded as indecisive.† It is uncertain whether dissolved haemoglobin is eliminated by the glomerular or the tubular epithelium: in the former case the granules, which contain iron and are evidently derived from haemoglobin, would be deposited or precipitated in the urine in the tubules and would increase in number, perhaps also in size, as the renal pelvis was approached; in the second case the granules would presumably be formed in the renal epithelium and would then be discharged into the renal tubules, without any subsequent increase in size, though they would become more closely packed if water were removed from the lumen of the tubules by the action of the renal epithelium. Our observations on Cases 7a, 11 and 16 (Figs. 33-44, and 53-58) do not enable us to assert that any increase in the size of the granules takes place as the collecting tubules are approached. In the latter, coarse masses of stainable material were encountered more often than definite granules. Possibly some change in the solid material contained within the tubules occurred during the time elapsing between the cessation of haemoglobinuria and death. In the kidney of the rabbit, in experimental haemoglobinuria (Figs. 45-51 and 59-60), no unmistakable increase in size of the granules could be recognised as the collecting tubes were approached, but only a more dense packing of the granules was observable. Several authors have, however, described an increase in size of the

\* By this it is not meant to imply that laking necessarily occurs in the peripheral circulating blood. What is meant is a haemoglobinaemia not secondary to renal haemorrhage.

† Ponfick, *Experimentelle Beiträge zur Lehre von der Transfusion*, Virch. Arch., 1875, B. 62, S. 273 (cp. Plate), concluded that dissolved haemoglobin was eliminated by the renal glomeruli and red cell fragments by the epithelium of the renal tubules. Marchand, *Zur Kenntniss der feineren Veränderungen der Nieren bei Hämoglobinausscheidung*, Virch. Arch., 1883, B. 91, S. 267, who describes a diapedesis of red cells, also adopts this view.



granules towards the distal tubules, both in blackwater fever and in haemoglobinuria due to potassium chlorate and other haemolytic agents. In these cases it may be questioned if the small and large granules in the proximal and distal portions of the renal tubules respectively are of contemporary origin. If the earlier granules, which as secretion of urine proceeds pass along the uriniferous tubules towards the collecting tubules, are large, and those formed later, when haemoglobinuria is passing off, are small, then the latter, by remaining in the upper portions of the tubules, might suggest an apparent increase in size of the granules as they descended towards the renal pelvis.

The presence of epithelial nuclei in the granular casts contained in the renal tubules, and later voided in the urine, supports the hypothesis that haemoglobin is eliminated by the tubular epithelium, which would appear sometimes to suffer a certain amount of degeneration in the process.

In Case 7a (Figs. 29 and 32) the individual granules were of exceptionally large size. Granules nearly as large, but much less densely packed together, were sometimes met with in rabbits during experimental haemoglobinuria (Figs. 22 and 23). Obviously suppression would be most likely to occur when the granules were of large size and numerous, especially if at the same time the amount of water in the urine were diminished in amount as this fluid passed along the uriniferous tubules.

3. The occurrence of a primary haemoglobinaemia would equally well account for the relapses occurring in blackwater fever as for the original attack (cp. Case 10, Table 50). In reference to this, it may be observed that the relapses of short duration, such as occurred in Case 10, are paralleled by the haemoglobinurias of short duration induced experimentally (cp. Exp. 10, Table 35, Fig. 9.)

4. On the other hand the presence of red blood cells in the urine in blackwater fever does not stand in any direct relation to haemoglobinaemia. Putting aside Case 2, which will be referred to separately later, the appearance of red cells appears to be due to the separation from the basement membrane of the epithelial cells of the uriniferous tubules which is observable in cases of suppression and which is indicated by the presence of epithelial casts in the urine in

non-fatal cases of blackwater fever. When granular casts are detached with separation of epithelium (Fig. 32) the possibility of a few red cells passing from the blood capillaries adjacent to the exposed basement membrane is always present and presumably accounts for their occasional presence in small numbers in blackwater fever.

5. In the experiments on rabbits recorded in Table 35 it was observed that the blood plasma after haemoglobinaemia and haemoglobinuria had passed off, was darker in colour than before experiment, though not so dark as in blackwater fever. Nevertheless yellowness of the sclerotics was not observed in experimental haemoglobinuria in rabbits. It would appear, however, that the orange or brownish amber-colour of the blood plasma was in both cases of similar origin, namely, due to the destruction of haemoglobin with the production of a brownish substance, which remains in solution, just as occurs when haemoglobin is broken up in a test tube under the action of hydrochloric acid or of quinine bihydrochloride.

6. The hypothesis of a primary haemoglobinaemia implies a preceding laking of red blood cells. So far, however, the site in which this laking may occur has not been determined (cp. pp. 150 to 160).

7. Haemoglobinaemia is, as such, unattended by pyrexia and general constitutional disturbance. The source of these must therefore be sought in the cause of the haemoglobinaemia.

Turning now to the consideration of the other hypothesis, that of a primary renal haemorrhage the following points require to be taken into consideration.

1. At the onset we are met with a serious difficulty. If the haemoglobinuria of blackwater fever were due to glomerular haemorrhage, the blood escaping being usually, we will suppose, laked at once owing to the low specific gravity of the urine leaving the glomeruli, then to account for this haemoglobinaemia we must assume that the dissolved haemoglobin so formed in the glomerular cavity is subsequently in part absorbed by the epithelium of the uriniferous tubules and then finds its way into the blood plasma. In such a case the amount of dissolved haemoglobin passing into the blood plasma would sometimes be greater than that passing into the urine, for in Case 7a (p. 195), for example, the blood plasma which contained

0.74 per cent. of dissolved haemoglobin would receive more—probably considerably more—than 11 g. of haemoglobin,\* while the urine contained about 9 g. of haemoglobin. It is difficult to assume that in such cases so large an absorption of dissolved haemoglobin by the renal epithelium could take place, when it is borne in mind that in experimental haemoglobinaemia (Table 35, Figs. 2 to 9) the stream of haemoglobin is strongly in the opposite direction, even when the percentage of dissolved haemoglobin is considerable in the lumen of the uriniferous tubules and is relatively slight in the blood plasma, as in Exp. 10, Table 35 (Fig. 9). This difficulty appears to be an insuperable one. It must, moreover, be remembered that if haemoglobinaemia were established as a result of glomerular haemorrhage an additional production of haemoglobinuria would thereby result, as in experimental haemoglobinaemia, so that in such a case the elimination of dissolved haemoglobin by the kidney would be a double process.

2. The production of granular casts under this hypothesis would fall into the first of the two possible methods referred to on p. 144, that is to say that we may assume that the granules would be precipitated as the urine containing dissolved haemoglobin passed along the uriniferous tubules. Another explanation might, however, be offered, namely that the red blood cells escaping from the glomeruli were imperfectly laked and that the granules represented red cell debris still remaining. This point is scarcely worthy of serious consideration. It may be observed that the granules seen in the urinary deposit (Fig. 32) and in the sections of the kidney (Figs. 33-36 and 53-4) in Case 7a, and also sometimes in the urinary deposit in experimental haemoglobinaemia (Figs. 22, 23) reach as much as  $5\mu$  in diameter and then present a considerable resemblance to red blood cells. They differ from the latter in their darker colour and smaller size and the absence of the typical form of red cells, though the latter might perhaps be attributed to the circumstance that these granules are densely packed together. Nevertheless we think the last two points and the fact that every transition occurs between the granules seen in Fig. 32 and the smallest granules seen in Figs. 22, 26 and 31, negative the view that the granules in Fig. 32

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\* It is assumed that the patient's blood plasma measured 1500 c.c.



represent altered red cells. Another difficulty arises when the attempt is made to explain suppression of urine on the hypothesis of glomerular haemorrhage. Here it becomes necessary to assume that the glomerular haemorrhage is general throughout the kidney, for otherwise a certain number of uriniferous tubules would remain to carry on the work of the kidney. It is easy to imagine that malarial parasites may be present in the glomeruli and damage the capillary wall thus leading to haemorrhage, but the necessity of supposing that this is general throughout the glomeruli of both kidneys and does not occur elsewhere in the blood vessels of the kidneys (for as is pointed out below, interstitial haemorrhage is not met with) seems to be a fatal objection to the hypothesis in question. It may be observed in passing that a high percentage, namely 1.1 per cent. to 2.7 per cent. of haemoglobin in the urine was observed in Cases 7a and 11 in which suppression occurred, but examination of the urine voided at intervals of several hours does not afford a comprehensive idea of what is taking place in the kidney. This will be made clear at once if the varying amounts of urine collected from the bladder at short intervals in Rabbit 10, Table 35 (Fig. 9, p. 84), are considered. It is obvious that only when the urine is collected continuously, and its haemoglobin content determined at short intervals, can a clear idea of the limits of the variation of the rate of elimination of haemoglobin be obtained.

3. As far as the occurrence of relapses is concerned the hypothesis of a glomerular haemorrhage being the source of the haemoglobinuria of blackwater fever must be equally applicable to the relapses and the original attack. This hypothesis if applicable to the original attack should be sufficient to explain also relapses of slight extent, such as those seen in Case 10 (Table 50).

4. The appearance of red blood cells in the urine would be expected as an occasional, if not a frequent, event in glomerular haemorrhage. It might be expected that partially decolourised red cells and red cell stromata would be often met with, and that red cells which were perfectly healthy in aspect would be unusual. In most of the cases (Table 43) in which red cells were found in the urine in blackwater fever, the majority of these cells were normal in aspect.



5. Glomerular haemorrhage would account for the anaemia, sallowness, and watery condition of the blood observed in blackwater fever.

6. Considerable difficulty is, however, experienced when an attempt is made to reconcile the theory of glomerular haemorrhage with the pathological conditions observed in the kidney after death. Lesions in the glomeruli would be expected, and occasionally haemorrhage into the glomerular cavities extending along the uriniferous tubules, while sometimes blood would find its way in considerable quantities into the urine. Moreover, if we suppose the haemorrhage to be due to malarial parasites in the blood capillaries, these parasites would scarcely be likely to be confined to the glomeruli, but would be present in the blood vessels of the kidneys elsewhere and would cause interstitial as well as glomerular haemorrhage. Now the kidneys have been so far free from these pathological conditions. In fact the changes in the kidneys are exceedingly slight and are practically confined to the presence of granular material in the tubules. Other changes, such as degeneration of the renal epithelium are inconstant, and when present should be regarded as complications of blackwater fever or of malaria, and not as essential factors in the production of haemoglobinuria. It may be remarked that superficial haemorrhages were found beneath the capsule of the kidney and in the mucous membrane of the renal pelvis of Case 7a, but these were evidently of very recent date and must have occurred after the disappearance of the haemoglobinuria. This case cannot therefore be regarded as in any way supporting the hypothesis of glomerular haemorrhage. Case 2 (p. 178) which appears at first sight to give some support to this hypothesis also fails to do so, when examined more critically. As already mentioned red blood cells were present in the urine in considerable numbers in this case, so as to form, on standing, a layer visible to the naked eye. It was found, however, that the red cells were accompanied by a number of pus cells, there being one pus cell to about eight red cells, while the urine, which was neutral in reaction to litmus paper also contained bacilli when voided. When haemoglobin had disappeared from the urine it was found that pus cells and bacilli still remained, and the previous history left no doubt that both had been present before the attack of blackwater fever. It is clear, therefore, that in

this case it could not be asserted that the red blood cells came from the kidneys, but it is more probable, since cystitis was present, that the red cells came from the situation in which pus cells were being discharged. The difficulty of reconciling the hypothesis of glomerular haemorrhage with the pathological findings is so great that from this standpoint alone this hypothesis must be disregarded.

7. As regards pyrexia and associated constitutional disturbance both hypotheses stand on the same level. The symptom of haemoglobinuria, whichever hypothesis is adopted to explain its causation, does not in itself necessarily imply accompanying constitutional disturbance.

The conclusion which follows from the above discussion is that the hypothesis of haemoglobinuria in blackwater fever being due to primary glomerular haemorrhage is untenable; because this theory (1) would not account for the haemoglobinaemia which accompanies blackwater, (2) nor would it furnish a satisfactory explanation of the mechanism of suppression of urine, while (3) it is out of harmony with the pathological findings in cases of blackwater fever in which death has occurred on the day of appearance of haemoglobinuria. On the other hand the hypothesis of a primary haemoglobinaemia furnishes a satisfactory explanation not only of the pathological findings, but also of the main symptoms of blackwater fever, namely the appearance of haemoglobinuria and of granular casts in the urine, which the experiments on rabbits, recorded in Table 35, prove to be a necessary consequence of haemoglobinaemia in such degrees as were observed in blackwater fever cases (Table 34, p. 75). In the light of all these facts it may, therefore, be confidently asserted that the haemoglobinuria of blackwater fever is dependent primarily upon haemoglobinaemia and is not due to renal haemorrhage. The site of the laking of red cells, causing haemoglobinaemia, remains to be ascertained. The mechanism of the rise of temperature and accompanying symptoms is also left unexplained. The former problem will now be considered; the latter is not capable of elucidation in the present state of our knowledge.

Concerning the site in which laking takes place, there are a limited number of data available. Such laking may occur in the blood in the peripheral circulation or in internal organs.

Now it is well known that malarial parasites may be found in red blood cells before the onset of, and during, blackwater, and it is stated by Stephens\* that, either on the first day of blackwater or on

TABLE 46. Examination of blood films in cases of blackwater fever. Leishman's stain.

Case	Time	Malarial parasites	Pigmented leucocytes
1	2nd day	None found	None found
"	3rd day	"	"
"	4th day	"	"
"	7th day	"	"
"	15th day	"	"
2	1st day	"	"
"	2nd day at 9.0 a.m.	"	"
"	2nd day at 2.15 p.m.	"	"
"	3rd day at 2.30 p.m.	"	"
3	5th day at 3.30 p.m.	Very scanty	"
"	6th day at 6.0 p.m.	None found	"
"	6th day at 10.0 p.m.	"	"
"	7th day at 11.45 p.m.	"	Very scanty
"	8th day at 10.0 a.m.	"	None found
"	15th day	"	"
4	5th day at 10.0 a.m.	"	"
"	6th day	"	"
"	16th day at 9.30 a.m.	"	"
5	5th day at 1.0 p.m.	"	"
"	6th day at 10.0 a.m.	"	"
6	3rd day at 4.0 p.m.	"	"
6a	3rd day at 4.30 p.m.	"	"
7	4th day at 12.30 p.m.	—	—
"	7th day	None found	None found
7a	1st day at 10.0 a.m.	"	"
8	5th day at 1.45 p.m.	"	Very scanty
"	6th day at 10.0 a.m.	"	None found
9	8th day at 10.0 a.m.	"	"
10	2nd day at 11.0 a.m.	"	"
"	3rd day at 10.30 a.m.	"	"
"	5th day at 11.0 a.m.	"	"
"	6th day at 11.0 p.m.	"	"
11	3rd day at 7.0 p.m.	"	"
"	4th day at 10.0 a.m.	"	"
"	7th day at 10.30 a.m.	"	"
12	2nd day at 4.30 p.m.	"	"
"	3rd day at 10.30 a.m.	"	"
13	—	—	—
14	4th day at 5.30 p.m.	None found	None found
"	5th day at 10.45 a.m.	"	"
14a	3rd day at 12.30 p.m.	"	"
15	1st day at 9.30 p.m.	"	"
"	2nd day at 10.0 p.m.	"	"
16	3rd day at 10.0 a.m.	"	"
17	1st day at 11.30 p.m.	"	"
"	2nd day at 9.0 a.m.	"	"
"	2nd day at 3.15 p.m.	"	"
"	3rd day at 10.30 a.m.	"	"
"	4th day at 11.45 a.m.	"	"

\* A System of Medicine, Clifford Allbutt and Rolleston, article Blackwater fever, London, 1907, vol. 11, pt. 2, p. 293.

the day preceding the attack, malarial parasites have been shown to be present in the majority of cases, that is in 62 per cent. to 96 per cent. of the attacks investigated, but it is also well known that the actual percentage of red cells, which can be observed to be affected, is frequently very small. If, however, the haemoglobinaemia met with in blackwater fever were due to the haemolysis of red cells affected with malarial parasites, present in the peripheral blood, then the affected red cells would be in such numbers as to be easily recognised. For example, a condition of haemoglobinaemia in which the blood plasma contained 1 per cent. of dissolved haemoglobin (cp. Obs. 38, Table 34, for example) would necessitate a minimum affection of one red cell in every hundred, while if this degree of haemoglobinaemia were prolonged for several hours the proportion of red cells affected would be considerably greater. In the cases of blackwater fever coming under our observation (Table 46) parasites\* were found in the peripheral blood on the day before the appearance of blackwater in one or two cases; on the first day of blackwater in not one out of nineteen observations (ten attacks); and in no observation made during blackwater fever at a later date. Observations of this kind are, however, inconclusive (and the same objection applies to spleen smears about to be described), for the condition observed cannot safely be assumed to represent that present at the onset of the attack. What is required, in investigating the situation in which laking may occur, is a quantitative examination of the distribution of malarial parasites in the red cells made just before haemoglobinaemia commences. In those attacks in which haemoglobinuria is the direct sequence of a single dose of quinine the time at which haemoglobinaemia commences can be determined. It is, however, only in exceptional cases that observations are likely to be made at this time, since in the majority of attacks the first indication of blackwater fever is the condition of the urine. It follows, therefore, that the data necessary to determine the relation of blackwater fever to malarial parasites contained in the peripheral blood will be obtained with considerable slowness. Until these data are obtained it will not be possible to make any definite statement as to the dependence of blackwater upon haemolysis, in the peripheral blood, of red cells

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\* About five thousand red cells were examined in each film.



containing malarial parasites. On one occasion (Case 2, Table 46) it happened that an examination of the blood was made within two hours of the establishment of haemoglobinuria and one hour before the administration of quinine. In this case no parasites could be recognised after careful search. It can be asserted, therefore, that in this case haemoglobinuria was not dependent upon the haemolysis, in the peripheral blood, of red cells containing malarial parasites. It is not, however, permissible to draw a general conclusion, applicable to all cases of blackwater fever, from this single instance. In passing it may be observed that the assumption, which is frequently encountered in the literature of blackwater fever, that considerable (that is to say, up to 20 per cent. or more) destruction of red cells occurs in the peripheral blood during an attack of blackwater fever, an assumption based upon red cell counts per cubic millimetre made before and after the attack, has not yet been established, because the total volume of the blood has not been determined in these cases and the influence of any accompanying variation in the volume of blood plasma thereby determined, nor has the high degree of haemoglobinaemia which would be expected to result from such extensive red cell destruction been met with in our observations. It may be added that in the blood films referred to in Table 46, made during and immediately before haemoglobinuria, the red cells were natural in aspect, no partially decolourised cells or stromata being seen.

If, instead of the peripheral blood, the blood in internal organs is considered, data of two kinds are available, the one relating to the enlargement of the spleen in blackwater fever, the other relating to the presence of malarial pigment or, less frequently, malarial parasites in the blood vessels of internal organs. Splenic enlargement is a common feature in attacks of blackwater fever. In the twenty attacks coming under our notice the condition of the spleen is given in Table 47. This organ was found not to be appreciably enlarged in one case (7) in which the attack of blackwater fever was slight, though two months later its lower border projected one and a quarter inches below the costal margin; in four attacks the condition of the spleen was not recorded (6a, 13, 14a, 15), but in two of these cases it was observed to be enlarged two months earlier (6a, 14a); in the remaining fifteen attacks the spleen was enlarged, slightly in four attacks and markedly, that is to say projecting one to four inches

below the costal margin, in the remaining eleven attacks. The illness was severe in those attacks in which the enlargement of the spleen was greatest; in those in which the enlargement was slight or not appreciable (7, 12, 14, 16, 17) the attack may be described as mild except in Case 16 (p. 235), in which death occurred seven days after the haemoglobinuria had ceased.

TABLE 47. Condition of spleen in blackwater fever.

Case	Spleen
1	Lower border projects $1\frac{1}{2}$ in. below costal margin.
2	" " $3\frac{1}{2}$ in. " "
3	" " 2 in. " "
4	" " $1\frac{1}{2}$ in. " "
5	" " 1 in. " "
6	Enlarged and palpable.
6a	(Two months later) condition could not be satisfactorily determined.
7	Not appreciably enlarged.
7a	(Two months later) lower border projects $1\frac{1}{4}$ in. below costal margin.
8	Enlarged and easily palpable.
9	Lower border projects 4 in. below costal margin.
10	" " $1\frac{1}{2}$ in. " "
11	Enlarged and easily palpable.
12	Slightly enlarged, just palpable.
13	Not examined.
14	Just palpable.
14a	(Three and a half months later) condition not recorded.
15	Condition not recorded.
16	Slightly enlarged.
17	" " just palpable.

The parenchyma of the spleen was examined by means of splenic puncture in six attacks (Table 48). This observation is readily carried out. The patient should be instructed to take a moderate inspiration and then hold his breath. A needle, about one millimetre in diameter, attached to a glass syringe, is then passed quickly through the abdominal wall into the substance of the enlarged spleen. The piston of the syringe is then drawn out a short distance, after which the barrel is detached, and the needle at once withdrawn. From the small amount of parenchyma in the point of the needle films are then prepared, and stained by Leishman's method. Care should be taken not to change the direction of the needle at any time during this procedure. After a little practice puncture can be carried out in about five seconds. Two of the smears obtained in Table 48

were made during haemoglobinuria (Obs. 4 and 5), and the remaining four cases at the close of haemoglobinuria or two to seven days later. In all cases the results were the same (Figs. 63-68). No malarial parasites were found, nor was malarial pigment observed. The red

TABLE 48. Condition of parenchyma of spleen in blackwater fever and malaria (Cp. Table 47).

No. of Observation	Illness	Spleen smear	Period at which examination was made	Relation to haemoglobinuria
1	Suppression of urine after blackwater, Case 7a	Phagocytosis of red cells (by less than 1% of the white cells). No malarial parasites nor pigment (Fig. 63).	On 9th day, 2 hrs. after death. Cp. Fig. 11.	Seven days after haemoglobinuria had ceased.
2	Suppression of urine after blackwater, Case 11	Phagocytosis of red cells (by less than 0.1% of the white cells). No malarial parasites nor pigment (Fig. 64).	On 6th day. Cp. Fig. 14.	Two days after haemoglobinuria had ceased.
3	Blackwater fever, Case 12	Phagocytosis of red cells (by 3% of the white cells). No malarial parasites nor pigment (Fig. 65).	On 2nd day. Cp. Fig. 15.	At close of haemoglobinuria.
3A	Blackwater fever, Case 12	Phagocytosis of red cells (by 3% of the white cells). No malarial parasites nor pigment.	On 3rd day. Cp. Fig. 15.	Twenty-four hours after haemoglobinuria had ceased.
4	Blackwater fever, Case 14	Phagocytosis of red cells (by less than 1% of the white cells). No malarial parasites nor pigment (Fig. 66).	On 4th day. Cp. Fig. 16.	During haemoglobinuria.
5	Blackwater fever, Case 15	Phagocytosis of red cells (by less than 1% of the white cells). No malarial parasites nor pigment (Fig. 67).	On 1st day. Cp. Fig. 18.	During haemoglobinuria.
6	Blackwater fever, Case 16	Phagocytosis of red cells (by about 1% of the white cells). No malarial parasites nor pigment (Fig. 68).	On 3rd day. Cp. Fig. 19.	Towards close of haemoglobinuria.
7	Malaria	Phagocytosis of red cells (by about 0.1% of the white cells). No malarial parasites nor pigment (Fig. 69).	In morning, T. normal (T. 103° F. on same and preceding evenings.)	—
8	Malaria	No phagocytosis of red cells. No malarial parasites nor pigment.	In morning, T. normal (T. 102° F. in evening).	—

cells were normal in aspect. The only change recognisable was phagocytosis of red cells on the part of the mononuclear leucocytes. This condition, to which attention was directed by Christophers and Bentley,\* was found to be slight in degree, the number of leucocytes

\* Note on the phagocytosis of red blood corpuscles in the spleen of a case of Blackwater Fever, Indian Medical Gazette, 1908, p. 81; Blackwater Fever, Scientific Memoirs by Officers of the Medical and Scientific Departments of the Government of India, No. 35, Simla, 1908, p. 51.



FIG. 63. Spleen smear, Blackwater Fever, Case 7a. Made at autopsy seven days after cessation of haemoglobinuria (succeeded by suppression of urine). To the left is seen a mononuclear leucocyte containing a red cell, which retains its haemoglobin unaltered. To the right a cell with two nuclei and an ingested red cell, whose haemoglobin is unaltered. Leishman's stain.  $\times 1,000$ .

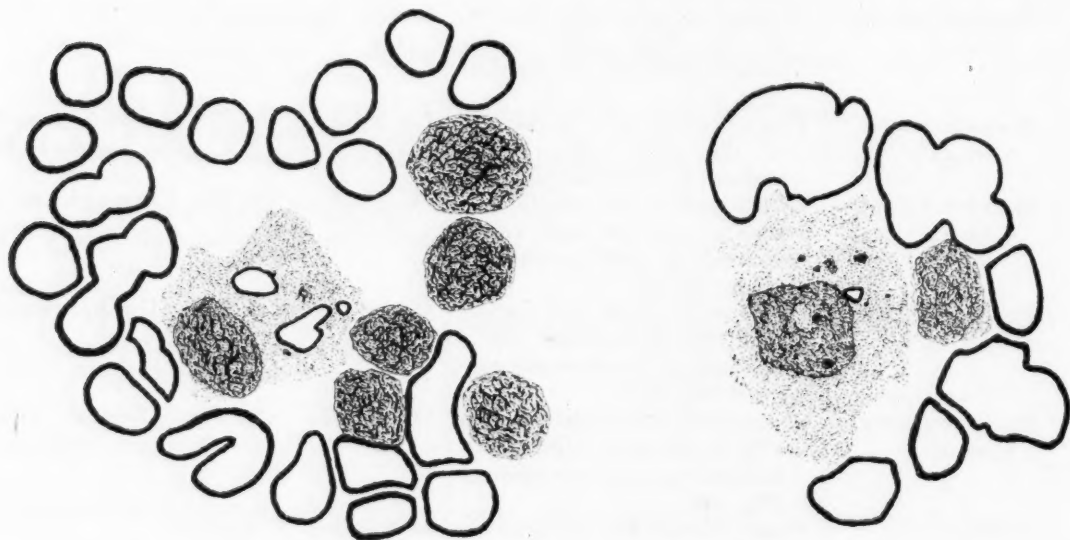


FIG. 64. Spleen smear, Blackwater Fever, Case 11. Made two days after cessation of haemoglobinuria (succeeded by suppression of urine). To the left a large mononuclear cell with abundant cytoplasm in which are contained three fragments of red cells retaining their haemoglobin; five lymphocytes are seen, as well as numerous free red cells. To the right a mononuclear cell with abundant cytoplasm, in which is seen a single red cell fragment, the haemoglobin of which is preserved; still further to the right a lymphocyte; around are red cells. Leishman's stain.  $\times 1,000$ .



containing red cells being usually considerably less than 1 per cent. of the whole number present (Table 48). It is not confined to blackwater fever. It was met with in spleen smears in one out of two cases of malaria (fig. 69) and in the circulating blood of a calf affected with piroplasmosis (fig. 70).

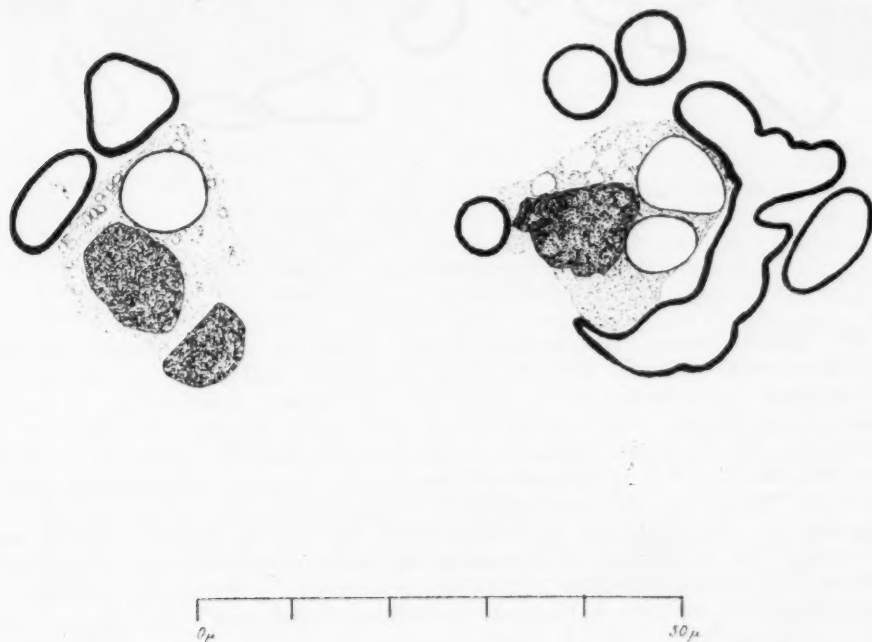


FIG. 65. Spleen smear, Blackwater Fever, Case 12. Made at the close of haemoglobinuria. To the left is seen a mononucleated cell in the cytoplasm of which is contained a partly decolourised red cell; above and to the left are two red cells, below and to the right is a lymphocyte. To the right is shown a large mononucleated cell, the cytoplasm of which contains two decolourised red cells; around are red cells, single and grouped. Leishman's stain.  $\times 1,000$ .

The presence of malarial pigment in the blood-vessels of the organs, usually the kidneys, has been described by several authors. In the three cases in which we had an opportunity of studying this point (Cases 7a, 11 and 16) no pigment was found in the kidneys, spleen or liver, nor could malarial parasites be recognised in the blood-vessels of these organs.

By way of summing up the above observations, it may be said that there are not at present sufficient data available to indicate where laking of red cells, causing haemoglobinaemia and thus leading to haemoglobinuria, takes place in blackwater fever.

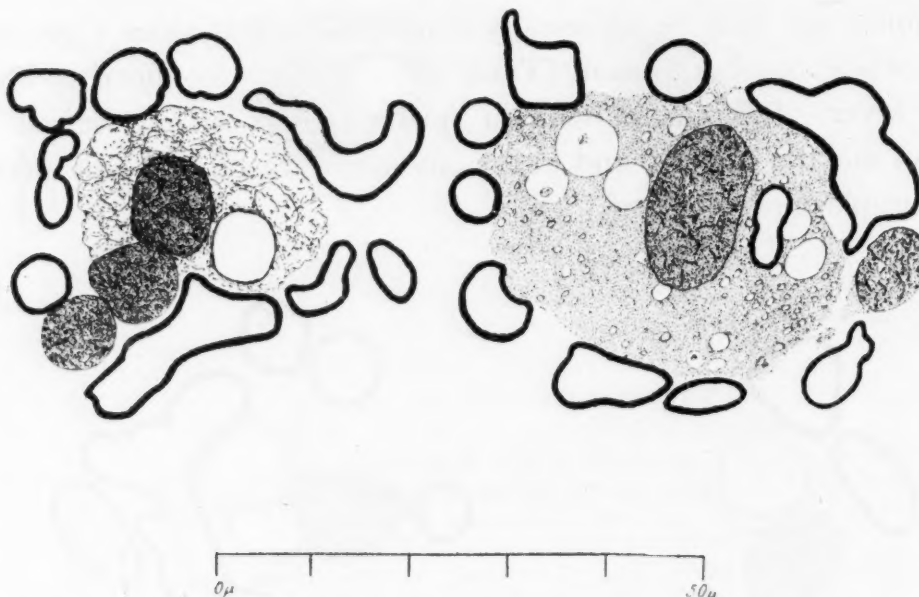


FIG. 66 Spleen smear, Blackwater Fever, Case 14. Made during haemoglobinaemia. To the left three mononuclear cells, one of which has abundant cytoplasm of a reticular character, containing a partly decolourised red cell; around are red cells, single or adherent in groups. To the right is seen a large mononuclear leucocyte surrounded by red cells; in the cytoplasm of the former is seen towards the right a red cell retaining its haemoglobin; immediately adjoining the nucleus on the left is a vacuole, the contents of which are stained slightly red, representing apparently the remains of an ingested red cell; in addition there are present in the cytoplasm numerous colourless vacuoles of varying size, usually with clear contents. Leishman's stain.  $\times 1,000$ .

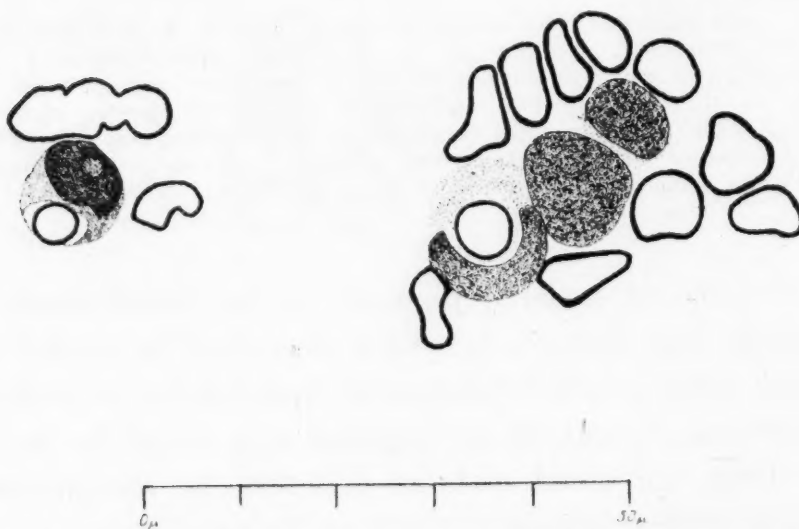


FIG. 67. Spleen smear, Blackwater Fever, Case 15. Made during haemoglobinuria. To the left is seen a mononuclear lymphocyte, in the cytoplasm of which is a red cell which retains its haemoglobin and is surrounded by a clear space; above and to the right are red cell masses. To the right are seen three nuclei, one of which has abundant cytoplasm, in which is contained a red cell whose haemoglobin is preserved unchanged; numerous free red cells are seen around. Leishman's stain.  $\times 1,000$ .

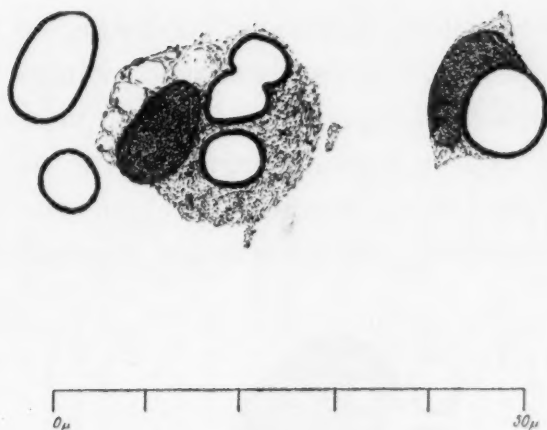


FIG. 68. Spleen smear, Blackwater Fever, Case 16. Made towards end of haemoglobinuria. To the left is a mononuclear cell with abundant cytoplasm, in which are three red cells with unchanged haemoglobin, two being in contact and the third separate; five vacuoles are seen in the cytoplasm; to the left are free red cells. To the right is a small mononuclear cell with scanty cytoplasm, containing a red cell, the haemoglobin of which is preserved unchanged. Leishman's stain.  $\times 1,000$ .

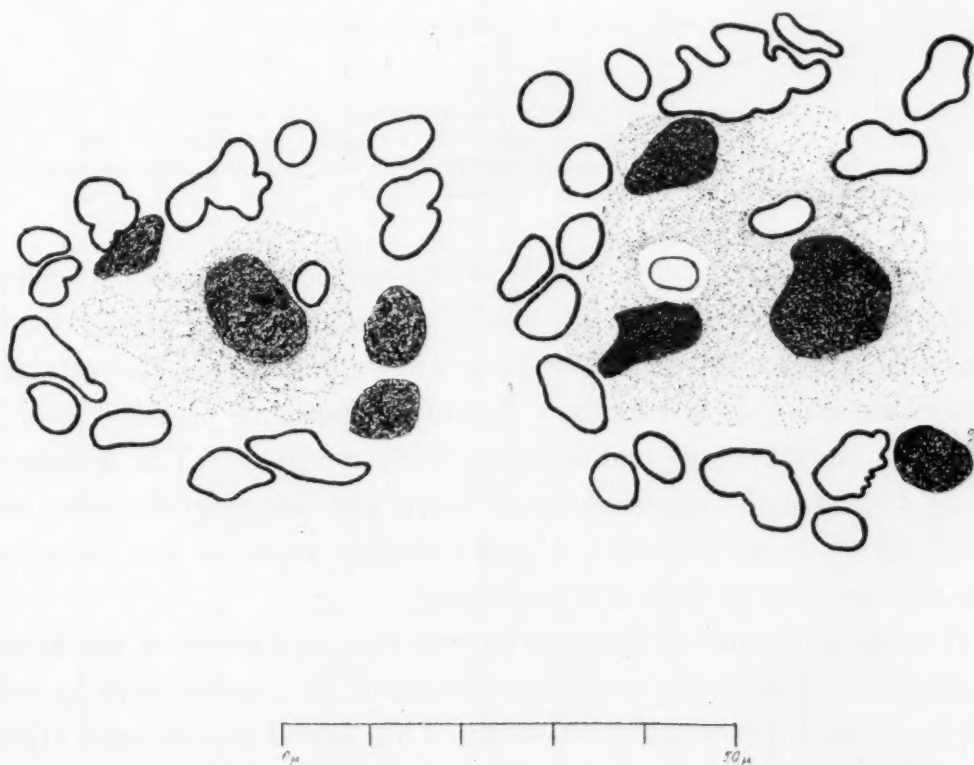


FIG. 69. Spleen smear, Malaria. To the left a large mononuclear cell with abundant cytoplasm containing a red cell with unaltered haemoglobin; around are three lymphocytes and numerous red cells. To the right are seen three nuclei, the cytoplasm of which has coalesced to form a single mass in which are two red cells, one of which (to the right) retains its haemoglobin, while the other, contained in a vacuole, is partly decolourised; around are numerous red cells, mostly in groups; below and to the left is a lymphocyte. Leishman's stain.  $\times 1,000$ .

Pyrexia and associated symptoms, consisting of rigor, sweating, loss of appetite, vomiting, malaise, headache, cramps in the legs, pain in the back, cardiac weakness, collapse, pain on micturition, and rapid anaemia, were met with in varying degree in the cases coming under our observation, as the clinical records show (pp. 176 to 256). Generally there was severe constitutional disturbance, especially at the onset of

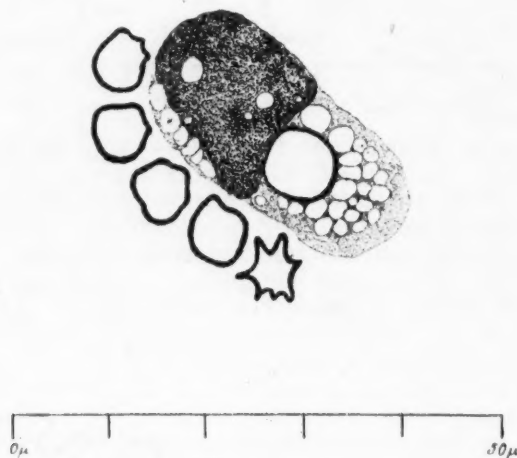


FIG. 70. Blood smear of calf with piroplasmosis. A mononuclear cell is shown, in the cytoplasm of which is a partly decolourised red cell together with numerous small vacuoles. Five free red cells are seen. Leishman's stain.  $\times 1,000$ .

haemoglobinuria, but subsequently, when haemoglobinuria had ceased, these symptoms gradually subsided. In sixteen out of the twenty observations recorded in Table 49 it will be noted that the attack of blackwater was ushered in by a rise of temperature usually reaching  $103^{\circ}\text{F.}$  to  $105^{\circ}\text{F.}$  and commencing with a rigor. The symptoms during the attack, regarded apart from the haemoglobinuria, were similar to those of malaria. Upon recovery more or less weakness, anaemia and loss of flesh still remained.

A striking feature of the attacks was that in sixteen of the twenty attacks investigated the onset was preceded for several days by more or less marked general disturbance. This lasted in fourteen attacks from one to seven days, and in two attacks ten and twenty-one days respectively, as will be seen on reference to Table 49. The preliminary illness was regarded as malarial, but in only one case (Case 3) were malarial parasites found before the onset of haemoglobinuria (they were sought for on the day before the onset in Cases 2 and 3).



TABLE 49. Character of constitutional disturbance during blackwater fever, and of preceding illness when present. Cp. with Table 50, p. 247.

No. of Attack	Malaria	Illness preceding attack of blackwater fever	Blackwater fever
1	Suffered much from malaria. Last attack one month ago.	No illness during fortnight before attack of blackwater fever.	Commenced with rigor, followed by haemoglobinuria. On second day vomiting, rise of temperature to 103° in evening. On third day subsidence of symptoms.
2	Has had several attacks of malaria during last eight years. Last attack many months ago.	Slight malaise for a week. On the day preceding blackwater suffered from pyrexia, vomiting, diarrhoea, and restlessness.	Commenced with rigor, followed by profuse perspiration, attended with blackwater; temperature 105° F. Next day improvement. On following day constitutional disturbance disappeared.
3	Has been almost free from malaria for last five years.	Malarial rigor five days ago, repeated three days ago, again two days ago, and also yesterday.	Commenced with shivering and vomiting; temperature 104°, attended with haemoglobinuria. Next day vomiting troublesome, temperature 102°. Better on following day. Symptoms then subsided.
4	Has suffered much from malaria during last seven years. Last attack about a year ago.	None. On thirteenth day again felt unwell, had pain in back, and vomited.	Commenced with headache, temperature 103° F., followed by haemoglobinuria. Still vomiting on second day. Headache on third day. Symptoms subsided by fifth day. On fourteenth day had a rigor, followed by haemoglobinuria; temperature 103° F. Next day symptoms subsided.
5	States that he has not had malaria.	Two days before appearance of haemoglobinuria had a rigor, followed by profuse sweating. Anorexia, but no vomiting.	Commenced with rigor and severe constitutional disturbance, attended with haemoglobinuria. Next day symptoms commenced to subside.
6	Has had much malaria during last fourteen years. Date of last attack uncertain.	Felt unwell for last four days, but continued at work until yesterday. Regarded his illness as due to malaria.	Commenced with severe constitutional disturbance, accompanied with haemoglobinuria. The latter disappeared on the third day. Vomiting was troublesome on third and fourth days. Symptoms subsided on fifth day.
6a	Last attack of malaria three weeks ago.	Felt unwell the day before the attack of blackwater fever.	Commenced to feel ill, temperature rose to 103.4° F., passed blackwater, vomited severely. Symptoms continued for next three days, then subsided on the fifth day.
7	Last attack of malaria six months ago. (Has had two previous attacks of malaria).	Felt ill three days ago, went to bed in afternoon. Next day felt unwell, but went to work, then had an attack of ague attended with shivering and profuse sweating. On the following day felt well in the morning, then vomited, and temperature rose to 104° F.	Passed blackwater, had pain in back and in hypogastrium, but otherwise no symptoms; temperature normal. Next day blackwater disappeared, and patient felt quite well.
7a	No attack of malaria subsequently to above.	Ten days ago felt unwell. Next day had a rigor, followed by profuse sweating, patient's temperature rising to 104° F. This illness, which was attributed to malaria, continued, the temperature remaining raised.	Rise of temperature to 105° F., accompanied with haemoglobinuria, profuse sweating, and severe constitutional disturbance. Next day general condition much improved; haemoglobinuria ceased, but suppression of urine developed and vomiting continued until next day.
8	States that he has had much malaria. Date of last attack uncertain.	Felt ill during the last three days.	Had a rigor, temperature rising to 104.4° F.; passed blackwater, vomited. Next day some improvement. On the following day haemoglobinuria ceased. On the succeeding day slight relapse, after which symptoms subsided.

TABLE 49—continued

No. of Attack	Malaria	Illness preceding attack of blackwater fever	Blackwater fever
9	States that he has had malaria many times. Date of last attack uncertain.	Has been unwell for last three weeks, attributing his illness to malaria.	Has a rigor attended with blackwater and temperature of 104.2. Next day vomiting troublesome. Haemoglobinuria ceased in fourth day. Constitutional symptoms subsided on fifth day.
10	During last two years has had much fever. Last attack three weeks ago.	Three days ago suffered from sore throat. On the following day temperature rose to 102° F.	Commenced with rigor, temperature rising to 105° F., with accompanying haemoglobinuria and vomiting. Haemoglobinuria continued with numerous remissions for fifteen days. Cp. Table 51, Fig. 13, and Clinical Notes p. 207.
11	Has had much fever during last nine months.	Has been unwell for a week; was much worse the day before blackwater appeared.	Felt very ill, temperature rose to 103° F., haemoglobinuria appeared, was very restless, vomited. On the third day suppression of urine occurred.
12	During the last six months has suffered from malaria two or three times a month.	Commenced to feel unwell the day before blackwater appeared.	Commenced with a rigor, followed by haemoglobinuria. The latter, together with general symptoms had completely subsided on the third day.
13	From time to time had malaria. Date of last attack uncertain.	None.	Illness commenced suddenly with vomiting and rise of temperature to 104° F., attended with blackwater. Then hyperpyrexia and death on same day.
14	During last five years has suffered considerably from malaria. Date of last attack uncertain.	Has been feeling unwell of late. Became ill with temperature of 100° F. and severe headache the day before the onset of blackwater.	Commenced with severe rigor and vomiting, temperature rising to 105.4° F., with accompanying haemoglobinuria. Next day another severe rigor, temperature reaching 106° F. On the following day condition improved, and symptoms had subsided two days later.
14 <sup>a</sup>	Date of last attack uncertain.	None.	Commenced with rigor and vomiting, temperature rising to 101.4°; then haemoglobinuria appeared. On the second day vomiting continued. On the third day had a severe rigor and temperature of 102° F., vomiting and insomnia continuing. On the fourth day all symptoms ceased.
15	During last six months has suffered much from malaria. Date of last attack uncertain.	Has been unwell for last four days, troubled with anorexia and insomnia, and unable to work.	Haemoglobinuria appeared without rigor; no marked constitutional disturbance at first, but in evening slight vomiting and temperature of 103.6° F. On the second and third days temperature still raised. On the fourth day general condition much improved. On the fifth all symptoms ceased.
16	During last four years has suffered much from malaria. Date of last attack uncertain.	Has been unwell for last four days.	Passed blackwater this morning; was obviously very ill, temperature 101.6° F.; has severe vomiting and diarrhoea. Haemoglobinuria ceased on third day, but albuminuria appeared, and patient continued in a very weak condition till death on the tenth day.
17	For many years has had one or two attacks of malaria every wet season.	Was taken ill four days ago, temperature rising to 105° F. Vomited. Regarded his illness as malarial.	Illness continued, haemoglobinuria appeared, temperature 101° F. in evening, vomiting troublesome. Another rigor on the second day, temperature 105° F. Temperature became normal on the third day, and all symptoms ceased on the fifth day.

One of the patients suffering from blackwater fever (Case 5) denied that he had ever had malaria; he had, however, an enlarged spleen, the lower border of which projected about one inch below the costal margin. With this exception all the patients coming under observation with blackwater fever had in the past suffered from repeated malarial attacks (Table 49, Col. 2). Although the diagnosis appeared to have been in all cases purely clinical, there is no reason to doubt its accuracy (cp. Table 47).

In attempting to interpret the significance of the preliminary illness above referred to we are met with the difficulty that it is unfortunately impossible in the present state of our knowledge to identify malarial pyrexia, and thus to distinguish in all cases this form of pyrexia from non-malarial pyrexia occurring in malarial subjects. The difficulty is connected with our ignorance of the mechanism of production of malarial pyrexia. In the same way we have no means of ascertaining if the pyrexia of blackwater fever is itself of malarial origin or not. It is equally impossible in the present state of our knowledge to determine the significance of the association existing between blackwater fever and malaria.

In all cases of blackwater fever coming under our observation quinine was taken prior to the attack, which it directly preceded (Table 50, p. 247). The relation between the two was usually quite distinct, as for example in Cases 1, 2, 6, and 14a. The interdependence of the two is not, however, a simple one, for in all but five cases (1, 4, 6, 11 and 14) quinine had been administered for one or more days before the appearance of blackwater, but without effect as far as the production of blackwater was concerned. In Case 7a (p. 195) the administration of quinine was observed by the patient to be followed, some days before the attack of blackwater, by a sudden rise of temperature attended with constitutional symptoms closely resembling those accompanying a previous attack of blackwater fever, though no haemoglobinuria made its appearance. It will be noted also that in five cases (4, 8, 10, 12, 14a, Table 50) it was deemed advisable to give quinine shortly after the disappearance of blackwater. In two of these cases (12 and 14a) no relapse occurred. In two other cases (4 and 8) slight relapses occurred, which did not bear any definite relation to the time of administration of quinine. In the remaining case (10) a remarkable series of slight relapses occurred,



at first in the absence of quinine, and then still continuing when, after a few days, the administration of quinine was recommenced, without, however, presenting any definite relation to the time of administration of quinine. In this case the administration of quinine did not appear to call forth the relapses, nor did any marked improvement occur during the period when quinine was discontinued.

It has already been pointed out that a possible explanation of the occurrence of haemoglobinuria after quinine in malarial subjects may be the destruction of the red cells, which, as already shown, are capable of taking up quinine from solution (Table 21, p. 41), by the double action of the malarial parasite and quinine, but that it is doubtful if the number of parasites in the red cells of the peripheral blood is large enough to admit of the assumption that the haemoglobin passing into solution in the blood plasma is derived from the haemolysis of these affected red cells, while we are without data pointing conclusively to the occurrence of haemolysis in the spleen or elsewhere. It must, however, be observed that we are not on safe ground in assuming that because quinine is effective in producing blackwater in malarial subjects it would fail to do so in non-malarial patients. Such an assumption is vitiated by the fact that the administration of quinine in large doses is in general use only in the treatment of malaria. In no other disease is the administration of this drug an habitual and generally recognised mode of treatment. Moreover, on the relatively few occasions on which quinine is administered in other diseases it is usually given in much smaller doses ( $\frac{1}{2}$  gr. to 2 gr.). Whether haemoglobinuria would occasionally occur if quinine were employed generally in pyrexial affections in doses similar to those employed in malaria, is purely a matter of conjecture.

The above remark is prompted by a point of resemblance between many cases of blackwater fever and the haemoglobinuric attacks produced by haemolytic serum, potassium chlorate and other drugs (p. 128). In the latter cases haemoglobinuria is induced in an animal which has for some time previously been suffering from more or less severe constitutional disturbance. Frequently this is also precisely the condition in which blackwater fever patients exist at or for some days before, the date of appearance of haemoglobinuria. In each case there are present two factors: more or less severe constitutional



disturbance, and a toxic agent. Another point of resemblance lies in the circumstance that in both cases blood destruction (changes in the red cells may be obtrusive after the administration of potassium chlorate) has been occurring previous to the appearance of haemoglobinuria resulting in more or less severe anaemia. In both cases an increased blood destruction occurs as soon as haemoglobinuria makes its appearance. It is, however, uncertain whether the blood destruction in these cases is attended with discharge of haemoglobin into the blood plasma, destruction or removal of haemoglobin (cp. Exp. 10, Table 35) occurring so rapidly that the degree of haemoglobinaemia is insufficient to lead to haemoglobinuria, or whether the destruction takes place without any such liberation of haemoglobin into the blood plasma. In the first case the difference between the first process and the haemoglobinaemia of blackwater fever would be only one of degree; in the latter case the difference between the two processes would be a qualitative one. In the former case quinine, potassium chlorate, etc., would act merely by accelerating the rate of discharge of haemoglobin into the blood plasma; in the latter case by inducing a new process. It is of interest to refer in this connection to the circumstance that malaria alone may cause blackwater fever. Twenty-four such cases are described by A. Plehn.\*

#### SUMMARY

1. In the urine in blackwater fever red cells may be found during haemoglobinuria, usually in very small numbers.
2. In blackwater fever the haemoglobinuria which occurs is the result of an accompanying haemoglobinaemia.
3. Sufficient data are not available to determine the situation in which laking of red cells leading to haemoglobinaemia occurs in blackwater fever.

#### ADDITIONAL NOTE

*Redwater in piroplasmiasis.* Haemoglobinuria in the dog, due to *piroplasma canis*, is similar in its mechanism of production to that of blackwater in the human subject, that is to say, the haemoglobinuria is attended with and dependent upon haemoglobinaemia. A series of data obtained in experimental piroplasmiasis of dogs will shortly be ready for publication.

\* Ätiologie und Pathogenese des Schwarzwasserfiebers, Virch. Arch., 1903, B. 174, S. 514.

## VI. THE CONDITION OF THE KIDNEYS IN BLACKWATER FEVER.

The condition of the kidneys during the haemoglobinuria of blackwater fever is of necessity a matter of inference, for a simple uncomplicated attack is not in itself fatal. When death takes place during blackwater fever, this is due to some complication, such as hyperpyrexia (Case 13), suppression of urine (Cases 7a and 11), or severe constitutional disturbance ending in cardiac failure (Case 16), and it may happen that haemoglobinuria has ceased some time before death. In these cases it is necessary to attempt to separate such pathological conditions as are purely representative of the haemoglobinuria from secondary or associated pathological conditions. The post-mortem appearance of the kidneys of animals in which experimental haemoglobinuria, together with severe blood changes, had been produced has already been referred to on p. 128, and our own experiments of a similar but simpler character, unattended with general constitutional disturbance, have been described on pp. 124-127 (figs. 45-51 and 59-60). It will therefore not be necessary to refer further to the appearances presented by the kidneys in these experimental conditions, but reference must be made to the results of post-mortem examination in cases of blackwater fever, omitting, however, any additional description of the kidney changes in suppression of urine following upon blackwater fever (pp. 107-124; figs. 33-40 and 53-56).

In 1882 Kiener and Kelsch\* in an article on '*Néphrite paludéenne*,' observed in Algeria, described as an early stage of this disease '*congestion hématurique ou hémoglobinurique*,' in which the urine contained sometimes dissolved haemoglobin together with brown granular casts and débris, in addition to hyaline casts, leucocytes and red cells, sometimes only red cells, but no dissolved haemoglobin. On section of the kidneys similar casts were found in the uriniferous tubules (loc. cit., p. 285-6; also Plate VI, figs. 8-14). The condition in question would now be called blackwater fever. Further investigations on the histology of the kidneys in blackwater fever were made

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\* Les altérations paludéennes des reins, Arch. der Physiologie, 1882, T. 14, p. 278.

by Stieda\* (1893), Marchiafava and Bignami† (1901), A. Plehn‡ (1903), De Haan§ (1905), and Werner¶ (1907). In some of the cases recorded, in which death took place on the day on which haemoglobinuria first appeared, it is possible if not probable that the condition of the kidneys would have led to suppression of urine if life had been prolonged. The changes to be considered may be grouped under four heads: the presence of granular material in the lumen of the renal tubules; degenerative and other changes in the renal epithelium; fluid distension of the renal tubules; interstitial changes. The first condition was always present in more or less marked degree. The state of the renal epithelium is not described alike by all observers, some finding the epithelium quite normal, others meeting with cloudy swelling, coagulation necrosis or fatty degeneration, or again the epithelial cells were found loaded with coloured granules. Distension of the renal tubules was usually absent; Werner|| describes four cases (Cases 4 and 5 and to less extent Cases 2 and 6) in which this condition was present, but these are not improbably cases in which suppression of urine would have been observed if the patients had survived. Interstitial changes (presence of epithelioid cells and leucocytes) were rarely met with.

In any attempt to apply the above observations to the elucidation of the enquiry, what is the condition of the kidneys in blackwater fever during simple haemoglobinuria, it is necessary to distinguish between the changes in the kidney which are necessarily present and those which when present are to be regarded as secondary or additional pathological changes. We are on safe ground when we infer, from the condition of the urine during blackwater and from post-mortem observations in blackwater fever and in experimental cases, that granular material is present in the renal tubules during haemoglobinuria whether dependent upon blackwater fever or produced experimentally. If haemoglobin is eliminated, not by the

\* Einige histologische Befunde bei tropischer Malaria, *Centralbl. f. a. Path. u. p. Anat.*, 1898, B. 4, S. 321.

† Malarial haemoglobinuria, *Twentieth Century Practice of Medicine*, London, 1900, Vol. 19, p. 483.

‡ Die Nieren beim Schwarzwasserfieber, *Arch. f. Schiffs- und Tropenhygiene*, 1903, Bd. 7, S. 270.

§ Die Nieren beim Schwarzwasserfieber, *Ibidem*, 1905, Bd. 9, S. 22.

¶ Über die Nieren beim Schwarzwasserfieber, *Ibidem*, 1907, Bd. 11, S. 5.

|| Loc. cit., pp. 9-11.



glomerulus but by the epithelium of the renal tubule, it is possible that this epithelium may necessarily contain during haemoglobinuria brown granules of haemoglobin as observed by Marchiafava and Bignami\* (cp. also Marchand† and Afanassiew‡). This is, however, on the whole, improbable; such an appearance seems to have been rarely met with, and was not observed in our experimental cases. In all probability the presence of granular material in the lumen of the renal tubules is the sole change recognisable to the naked eye, which the kidney presents during haemoglobinuria. As regards the degenerative changes occasionally observed in the renal epithelium after death, the circumstance that the urine ordinarily becomes quite normal after the attack of blackwater has ended, no trace of coagulable proteid remaining in the urine after haemoglobin has disappeared, shows that as far as mere haemoglobinuria is concerned the renal epithelium is unaffected, the elimination of haemoglobin being effected by healthy epithelium as occurs in the rabbit during experimental haemoglobinuria (Figs. 44-51 and 59-60). No parenchymatous change of the epithelium necessarily attends the appearance of haemoglobinuria, nor does the constitutional disturbance accompanying blackwater fever seem to be usually accompanied with any secondary affection of the renal epithelium. Whether the granular material in the renal tubules ordinarily causes some obstruction to the flow of urine is uncertain. No diminution, but an increased flow occurred in the experiments on rabbits detailed in Table 35. In blackwater fever patients, however, a diminished flow of urine may be observed not unfrequently during haemoglobinuria (cp. p. 134). The circumstance that interstitial changes are usually absent after death from blackwater fever may be regarded as negating the necessary association of this condition with the production of haemoglobinuria.

We may therefore say that all the evidence available points to the conclusion that the production of haemoglobinuria in blackwater fever is consistent with a normal functional state of the kidney, and is not

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\* Loc. cit.

† Über die Intoxication durch chloresaurer Salze, Virch. Arch., 1879, B. 77, S. 455.

‡ Über die pathologisch anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Icterus verbundenen Vergiftungen, Virch. Arch., 1884, Bd. 98, S. 460. Afanassiew regarded these granules as identical with the fragments of red cells seen in his experiments in the circulating blood; these he describes as being taken up by the renal cells and passed on into the lumen of the tubules. Marchand and Ponfick hold similar views.



attended with any pathological condition of the renal epithelium, the only visible change ordinarily present being the appearance of brown granular material in the renal tubules. Whether brown granular material is also of necessity present in the epithelium of the renal tubules during blackwater is uncertain.

It is moreover clear from our observations that an attack of haemoglobinuria does not necessarily damage the kidneys. No albuminuria of renal origin followed the attacks of blackwater fever coming under our observation, in which recovery occurred, and judging from the cases recorded in the literature of this subject, blackwater fever is not a cause of nephritis.

Before concluding this section reference may be made to the circumstance that venereal disease does not seem to predispose to blackwater fever nor to influence its course when present. Particulars on this point were obtained in sixteen cases of blackwater fever, with the result shown in Table 51. On further comparison of the cases in

TABLE 51. Blackwater fever and venereal disease.

No. of Cases	Syphilis	Gonorrhoea
9	Absent	Present in 3 cases
7	Present	Present in 3 cases

which one or both forms of venereal disease were present with those in which a negative history was obtained, it is found that the severity and duration of the blackwater fever were not obviously greater in the former than in the latter.

### SUMMARY

1. During simple uncomplicated haemoglobinuria of blackwater fever the sole pathological condition existing in the kidneys would appear to be the presence of brown granular material in the lumen of the renal tubules.

2. Venereal disease does not appear to influence either the tendency to blackwater fever or its severity when present.

## VII. ICTERUS AND BLACKWATER FEVER.

The statement is frequently made that icterus occurs in blackwater fever. This word, while in its strict sense designating true jaundice due to the presence of bile pigment in the blood plasma and urine, is also sometimes employed loosely to indicate merely a yellowish tinting of the skin and sclerotic coat of the eyeball, not necessarily due to bile colouring matter. The use of the term icterus in describing this latter condition in blackwater fever, without any statement as to whether the urine contains bile pigment or not, is frequent and is apt to be misunderstood. The same indefiniteness of meaning is also apparent in the description of experimental work in which haemoglobinuria has been induced. It would appear, however, that true jaundice, dependent upon bile pigment, is producible together with haemoglobinuria by toluylendiamin,\* though so far as we know no other drug is capable of producing these two conditions simultaneously. It is asserted by Koch† that after the administration of quinine, instead of blackwater fever, an attack of icterus may result, haemoglobin being converted into bile pigment, which appears in the urine.

In the cases of blackwater fever which came under our notice, as also in cases of malaria, the urine was not unfrequently high coloured (brownish amber) after the attack. When the urine obtained during the haemoglobinuria of blackwater fever was acidified and boiled, the liquid obtained on filtration was usually of a light amber colour, though sometimes remaining of a somewhat brownish tint. On testing the filtrate for bile pigment with iodine a negative result was obtained. In the same way a negative result was obtained in the experimental haemoglobinuria of rabbits. In consequence of the absence of bile pigment in the urine it follows that the dark amber or orange colouring matter, obviously derived from the breaking up of haemoglobin, which the blood plasma often presents in blackwater fever and for some time subsequently, and sometimes also in malaria (Table 33, p. 73) is not due to bile pigment.

\* G. Joannovicz, Experimentelle Untersuchungen über Ikterus, Zeitschr. f. Heilkunde, 1904, Bd. 25, S. 25. M. Afannassiew, Über die pathologisch-anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Icterus verbundenen Vergiftungen, Virch. Arch., 1884, Bd. 98, S. 460.

† Über Schwarzwasserfieber (Hämoglobinurie) Zeitschr. f. Hygiene, 1898, Bd. 30, S. 295. Cp. pp. 321-322 and Case 3, p. 303.

### VIII. REMARKS ON THE PROPHYLAXIS AND TREATMENT OF BLACKWATER FEVER.

It is not here proposed to do more than refer briefly to the importance of avoiding malaria in countries in which blackwater fever is prevalent, for the need of adopting the various measures available for preventing the risk of the bites of infected mosquitos and of using quinine as a prophylactic are now well recognised. But a few remarks upon the attitude often taken up towards prophylactic measures will serve to indicate the nature of some of the difficulties, often more or less trivial in aspect, which arise when it is attempted to secure avoidance of malaria. It was, we found, commonly regarded as unnecessary to use mosquito nets until mosquitos had already made their appearance. The presence of mosquitos was, moreover, not unfrequently overlooked. In many cases this appeared to be in part due to the circumstance that those bitten by mosquitos have after the lapse of months or years ceased to suffer from irritative skin lesions as a result of these bites. Mosquito nets, when available, were sometimes not used. Some of the nets in use were in a torn condition and of limited utility. When travelling or shooting mosquito nets were not unfrequently left behind, though the night would be spent in the neighbourhood of a river where mosquitos abounded, and this in spite of warnings afforded by the consequences of such neglect. The importance of taking a course of quinine as a prophylactic after being bitten by mosquitos is not recognised, and this drug is not taken until an attack of malaria has supervened, or until several malarial attacks of increasing severity have occurred. The necessity of avoiding the formation of collections of water in the neighbourhood of houses, or of filling up or draining places in which, during the rainy season, mosquitos may breed is generally unknown to the white population, but the Government medical officers have taken steps to secure better sanitary conditions in such cases. Many of the situations in which habitations were placed involved unnecessary risk of exposure to malaria.

The administration of quinine to a patient suffering from malaria who has previously suffered from blackwater fever presents a difficulty for which no general rule can be laid down. In some cases where the malarial condition is not showing signs of improvement, or is becoming steadily worse, the possible risk of inducing blackwater by



giving quinine may be less than that of withholding it. Sometimes the decision may be left to the patient, but in most cases the responsibility of deciding upon the treatment will necessarily rest with the medical officer. We are of opinion that the general avoidance of quinine in malarial cases with a view to escaping the possible risk of blackwater fever would expose patients to a much greater risk of fatal issue from complications of malaria. The degree of risk involved in giving quinine shortly after an attack of blackwater fever may be judged from a glance at Table 50 (Cases 4, 8, 12, 14a). It is scarcely necessary to add that when quinine is given its use should be continued as long as splenic enlargement or other sign of malaria is observable.

During the attack of blackwater fever quinine is probably best avoided, but when haemoglobinuria has ceased and the temperature has become normal, the treatment of the accompanying malarial condition may be commenced with  $\frac{1}{2}$  gr. daily doses of quinine; very small doses appearing to be much less likely to cause haemoglobinuria than larger doses. This amount may be gradually increased to 5 gr. a day (cp. Case 14a, Table 48).

Of the treatment of the general symptoms of blackwater fever nothing will be said, since these are dealt with by the same methods which are employed when they occur in other diseases. Our experience does not enable us to express any opinion as to the value of the numerous empirical methods for the treatment of blackwater fever which have been put forward by various authors. A few remarks may, however, be made on the treatment of a complication which is peculiar to blackwater fever and is a frequent cause of death, namely, suppression of urine. It has already been pointed out that the tendency to plugging of the renal tubules with granular material, which is purely mechanical in its action, appears less likely to occur when the flow of urine is rapid than when it is sluggish. For this reason in all cases of blackwater fever, and especially when the amount of urine secreted is small, which is likely to be the case when the patient has been sweating profusely, as often happens, or exhibits signs of cardiac depression, the secretion of urine by the patient should be encouraged by the administration of a copious amount of fluid to drink and by the use of tea, caffeine, digitalis or other diuretics. If suppression has supervened these



measures may be continued for a time, but if suppression continues the question arises whether any attempt to relieve the blocking of the renal tubes by providing an outlet mechanically by incision of one of the kidneys should be made. Should such a measure have been attempted in Case 7a, for example, in which the general condition was good almost to the end? Up to the present this procedure, first suggested by Werner,\* appears to have been adopted in suppression of urine in blackwater fever only on two occasions. In the first case,† on the third day of suppression nephrotomy was performed, the flow of urine containing abundance of albumin being re-established, but subsequently anuria reappeared and death occurred. In the second case‡ nephrotomy was performed after five days' suppression of urine; the operation resulted in the re-establishment of a profuse secretion of urine, but death occurred subsequently from progressively increasing weakness. It is clear, on reference to Fig. 62 (p. 130), that if the blocking of the renal passages is sufficiently extensive no mere incision of the kidney would be of much use, but if the blocking is chiefly confined to the ducts of Bertini, as probably occurs in most cases and was observed in Cases 7a and 11 (Figs. 33-40 and 53-56), then nephrotomy, if the patient's strength is maintained, offers a means of re-establishing the flow of urine. It does not, however, follow that this will necessarily lead to recovery, for though cases are on record in which after several days' suppression in blackwater fever re-establishment of the flow of urine has occurred spontaneously, yet, nevertheless, death occurred from other complications; two such cases are recorded by F. Plehn. The effect of incision is of course limited, as regards the outlet which it provides for the escape of urine, to only a small fraction of the kidney incised, but it may be pointed out that a fraction of one kidney not exceeding one half to one quarter of the total kidney mass is sufficient to carry on the work ordinarily performed by the two kidneys§. The best time for carrying out nephrotomy is probably before the suppression, determined by catheterisation, has lasted more than twenty-four to forty-eight¶ hours, it being, of course, assumed that the general condition of the patient is sufficiently good to permit of operation.

\* Ist bei Schwarzwasserfieberanurie die Nephrotomie indiziert? Deut. med. Wochenschr., 1901, No. 42.

† Ziemann, Monographie über Malaria, Mense's Handbuch der Tropenkrankheiten, Bd. 111, S. 585-6.

‡ Reported by Krüger. See Werner, Über die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- u. Tropenhyg., 1907, Bd 11, S. 5.

§ R. M. Pearce, The influence of reduction of kidney substance upon nitrogenous metabolism, Journ. of Exp. Medicine, 1905, Vol. 10, p. 632.

¶ Cp. Werner, loc cit., p. 17.

### IX. CLINICAL RECORDS.

Before giving the clinical records of the patients coming under observation, a few words may be said, by way of introduction, concerning the general features presented by the attacks of blackwater fever from which they suffered. For convenience of reference, a synopsis is given in Table 52, p. 248. The observations have already been for the most part collected in tabular form, as indicated below. Some of the particulars supplied to us by the patients, for example, the statements made as to past attacks of malaria, it was not in our power to verify.

The records are of necessity more or less incomplete. In some cases delay occurred in reaching the patient, either because information could not be sent to us sufficiently early, or, more frequently, because of the slowness of travelling, especially during the rainy season. The simultaneous occurrence of two or more cases at considerable distance apart caused difficulty also on more than one occasion.

The number of attacks of blackwater fever investigated was twenty. These occurred in seventeen different individuals, all males, eleven being white men, four Indians, one Eurasian and one Chinese. Only one attack occurred in a female during our stay in Nyasaland; this was, however, not brought under our observation.

For convenience of reference and comparison dates are omitted, and the course of the attacks is described under the headings 1st day, 2nd day, etc. (cp. Table 50, p. 247). One attack occurred in January, six in February, four in April, one in May, two in June, three in September, two in October and one in December. The rainy season extends from November to March.

All the patients, except Case 5, stated that they had suffered from malaria some time before the attack (cp. Table 49, p. 161). In all except four (Cases 1, 4, 13 and 14) of the attacks the patients were regarded as suffering from malaria before the appearance of blackwater. Malarial parasites were found in the peripheral blood on only one occasion (Case 3), as is indicated in Table 46 (p. 151).

In all the twenty attacks investigated quinine had been taken before the onset of haemoglobinuria, of which it appeared to be a

determining factor, especially in attacks 1, 2, 3, 6, 7, 11, 12, 13, 14 and 14a. This relationship is exhibited in tabular form in Table 50 (p. 247), and is further considered in Section V, pp. 136-165.

The duration of the attack of haemoglobinuria was usually short, lasting from three or four hours to four days. (Table 50). Relapses occurred in three cases (4, 8 and 10). In the first two a single relapse occurred respectively ten days and two days after the original attack; in the last case a remarkable series of small relapses, about fifteen in number, occurred during the ten days following the original attack, and bore no obvious relation to the quinine taken during this period (Table 50). Three of the patients (6, 7 and 14) suffered from another attack of blackwater fever after the lapse of a period varying from two to three and a half months.

The severity of the constitutional symptoms varied considerably in different attacks, being insignificant, for example, in Case 7, while severe collapse lasting about twenty hours occurred in Case 2. Vomiting was severe in attacks 3, 6a, 9, 14a, 16 and 17. Pain in the back was common, but was not severe. Rigors occurred at the onset of the attack in Cases 1, 2, 3, 5, 8, 9, 10, 12, 14 and 14a. Cramps in the calf muscles were severe in Case 2. Illness of several days' duration preceded the attack of blackwater, except in 1, 4, 13 and 14a.

The complications observed were hyperpyrexia (Case 13), suppression of urine (Cases 7a and 11) and vomiting attended with extreme weakness (Case 16). All these complications terminated in death.

The blood obtained by pricking the finger tip was generally remarkably watery in aspect. Haemoglobinaemia was usually present during haemoglobinuria in the cases in which this point was investigated (3, 7a, 10, 14, 15 and 17, cp. Table 34, p. 75), the highest amount observed being 0.95 per cent. Methaemoglobin was also observed in relatively small amount during blackwater in most cases in the blood plasma, which was dark-orange in colour in every attack, except 5, 6a and 9, in which it was light yellow (Cases 1 and 13 were not examined)

The reaction of the red blood cells to quinine in vitro was noted in Cases 3, 4, 5, 7, 7a, 8, 10, 11, 14, 14a and 15; it was found not to exhibit any marked alteration (Table 24, p. 46).



The percentage of haemoglobin in the urine lay, in the cases in which this could be determined, between 0·2 per cent. and 3·8 per cent. (Table 43, p. 136). The total amount of haemoglobin, lost in the urine, ranged between 1·5 g. and 75 g. (Table 45, p. 141). The total amount of dissolved haemoglobin entering the blood plasma could not be determined (cp. p. 143). Methaemoglobin was also usually observed in the urine in relatively small amount. Red blood cells were found in the urine (Table 43). Casts, generally granular in character, were always present (Section IV, p. 94; Figs. 21 to 32). The urine before and after the attack was frequently high coloured, as in Cases 2 and 7, for example. Bile pigment was not found in the urine.

In Cases 7a and 11 (suppression of urine) and in Case 16 (death seven days after blackwater had ceased) some of the renal tubules, in particular the collecting tubules, were found to be plugged with coarsely granular casts (Figs. 33-40 and 53-56) resembling those present in the urine in Case 7a (Figs. 29 and 32). The renal epithelium did not show any marked degenerative changes. The condition of the urine during nine days' suppression is given on p. 199 (Case 7a) and on pp. 220-221 (Case 11).

The spleen was observed to be enlarged, usually markedly so, in all cases except 13 and 15, in which no information as to its condition was available (Table 47, p. 154)

#### BLACKWATER FEVER. CASE I.

Male, twenty-nine years of age. Engineer. European.

Has lived in Nyasaland for the last five years. During this period he has suffered a good deal from fever. Has had one previous attack of blackwater fever about three years ago. He does not take quinine regularly. About a month ago he suffered from low intermittent fever. He stated that he had an attack of fever each alternate afternoon about 3 p.m. These relapses went on for about a fortnight, and during this time he took no quinine. After these attacks of fever had stopped, he took five grains of quinine daily for three or four days. During the next fourteen days he felt very well, and took no quinine



On the 1st day of the present illness he got up feeling very well, and took five grains of quinine. About noon he suddenly became dizzy and had a rigor, and shortly afterwards he passed blackwater.

2nd day. In the morning patient was comfortable, vomiting was slight, and the urine, which had begun to clear about noon, was still deeply tinged red. Pulse 84. Temperature normal. The spleen extended one and a half inches below the costal margin. The liver was also enlarged. At 9 p.m. patient was not quite so well. Temperature 103° F. The urine had become much darker again.

3rd day. Patient much better; the vomiting had ceased and the urine was not so deeply coloured. Temperature 99.5° F.

4th day. Patient better; the urine was amber coloured and the temperature normal. Subsequently the patient's recovery was uninterrupted.

*Condition of blood.*—On the 2nd day an examination of the patient's blood was made at 4-30 p.m. No malarial parasites and no pigmented leucocytes were found.

3rd day. Blood was examined at 10 a.m. No malarial parasites and no pigmented leucocytes found.

4th day. Blood films were examined again to-day, and no malarial parasites or pigmented leucocytes were found.

7th day. No malarial parasites or pigmented leucocytes were found in films made from the patient's blood to-day.

15th day. No isolysin could be discovered in patient's blood plasma. No malarial parasites or pigmented leucocytes were found in the blood films.

*Condition of urine.*—On the 1st day, at 2 p.m., the urine was of a dark red colour.

2nd day. The urine was not quite so deeply coloured at noon to-day, but at 9 p.m. it became of a dark red colour.

3rd day. The urine was of a reddish colour to-day, but became lighter in the evening. On spectroscopic examination the bands of oxyhaemoglobin were seen. Methaemoglobin bands were absent. A few red cells were present in the urine.

4th day. Urine was amber coloured.

15th day. The urine was acid, sp. gr. 1.010, amber coloured; no deposit on centrifugalising, and no precipitate on acidifying and boiling.

## BLACKWATER FEVER. CASE 2.

Male about forty years of age. Planter. European.

Has been in Nyasaland for thirteen years. His first attack of blackwater fever occurred eight years ago; was ill with 'fever' for a fortnight, then took forty grains of quinine, after which blackwater appeared. Since then has had several attacks of malaria, but has not suffered again from blackwater fever. Has passed small amounts of pus in his urine for last twelve months, and occasionally has passed bright blood at the end of micturition.

Eight days ago was drenched, and could not change his clothing for some hours. Yesterday took five grains of quinine, had to pass a catheter, being unable to void urine naturally.

To-day (first day) temperature became raised; patient suffers from vomiting and diarrhoea, and is very restless. Has painful cramps in muscles of legs. Took five grains of quinine.

2nd day. Still restless but somewhat improved in morning. At 10 a.m. had six grains of quinine bihydrochloride hypodermically, his temperature being then  $98.4^{\circ}\text{F}$ . At 11-15 a.m. again restless, temperature  $103.5^{\circ}\text{F}$ ., vomited. At 12-0 mid-day passed 150 c.cm. of very dark chocolate-coloured urine. Pulse now very feeble and rapid; shivering; extremities cold. At 12-30 p.m., temperature  $105^{\circ}\text{F}$ .; at 1-30 p.m., temperature  $101^{\circ}\text{F}$ . Then commenced to perspire freely, and condition improved. Spleen projects about  $3\frac{1}{2}$  in. below costal margin. Liver cannot be felt. Heart and lungs normal.

3rd day. Condition much improved. Skin has become sallow. Urine contained chocolate-coloured deposit until mid-day, when this disappeared and dark amber urine was passed.

Subsequently patient's condition steadily improved. The urine continued free from haemoglobin, no relapse occurring. Pus in urine continued as before.

*Condition of blood.*—On the 1st day, before blackwater appeared, an examination of patient's blood was made at 4 p.m. The blood plasma was of a dark orange colour with a slight greenish tint, and gave, in a column 18 mm. high, excessively faint oxyhaemoglobin bands, the amount of dissolved haemoglobin being about 0.13 per cent. No isolysin was present. No malarial parasites could be found in blood smear.

2nd day. Blood examined at 9 a.m. and again at 2-15 p.m. On each occasion the plasma was found to be of a dark orange colour, with a slight greenish tint, containing about 0.13 per cent. of dissolved haemoglobin. No isolysin present. No malarial parasites in blood smear.

3rd day. Blood examined at 2-30 p.m. Plasma of dark orange colour, with slight greenish tint. As before, only excessively faint oxyhaemoglobin bands could be recognised in a column 18 mm. high, the amount of dissolved haemoglobin being about 0.13 per cent. No isolysin present. No malarial parasites in blood smear.

*Condition of urine.*—Prior to the appearance of blackwater the urine was stated to be of a yellow colour; none was obtainable before the onset of blackwater. Characters of urine are given in the following table:—

Date.	Amount.	Specific Gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination.
2nd day, 12.0 mid day	80 c.c.	1.022	Neutral	Chocolate coloured	5 c.c. chocolate brown	No dissolved haemoglobin recognisable
" 6.45 p.m.	50 "	1.019	"	"	7 " "	" "
" 11.30 "	118 "	1.022	"	Dark amber coloured	3.5 c.c. "	" "
3rd day, 3.20 a.m.	125 "	1.012	"	"	1 c.c. whitish	" "
" 6.30 "	85 "	1.014	"	"	2 " "	" "
" 9.30 "	136 "	1.014	"	"	3 " "	" "
" 12.0 p.m.	115 "	1.018	"	"	6 c.c. "	" "
" 3.30 "	115 "					
" 4.15 "	60 "					
4th day, 2.0 a.m.	170 "					
" 3.45 "	85 "					
" 6.30 "	195 "	1.018	"	"	6 c.c. "	" "
" 9.0 "	210 "					
" 11.45 "	220 "					

The first two specimens of urine presented an abundant deposit, consisting chiefly of red blood cells and stromata, and a dark brown granular precipitate, the latter being one third to one quarter of the volume of the former. In addition a few epithelial cells were seen, and also bacilli, mostly  $3\mu$  to  $4\mu$  long, somewhat less numerous than the red cells. On centrifugalising the urine, the brownish precipitate was separated with difficulty, and a turbid upper liquid, light brown in colour, showing on spectroscopic examination no haemoglobin bands in a layer 18 mm. high, and giving on acidifying and boiling about



one-twentieth column of a whitish brown precipitate, was obtained. From the supernatant liquid no haemin crystals could be obtained, but on adding tincture of guaiacum and hydrogen peroxide a blue colour was obtained. The deposit in the second specimen of urine contained in addition a large number of pus cells (about one to every eight red cells), some of which had ingested red cells, and a few blood casts and brown granular casts.

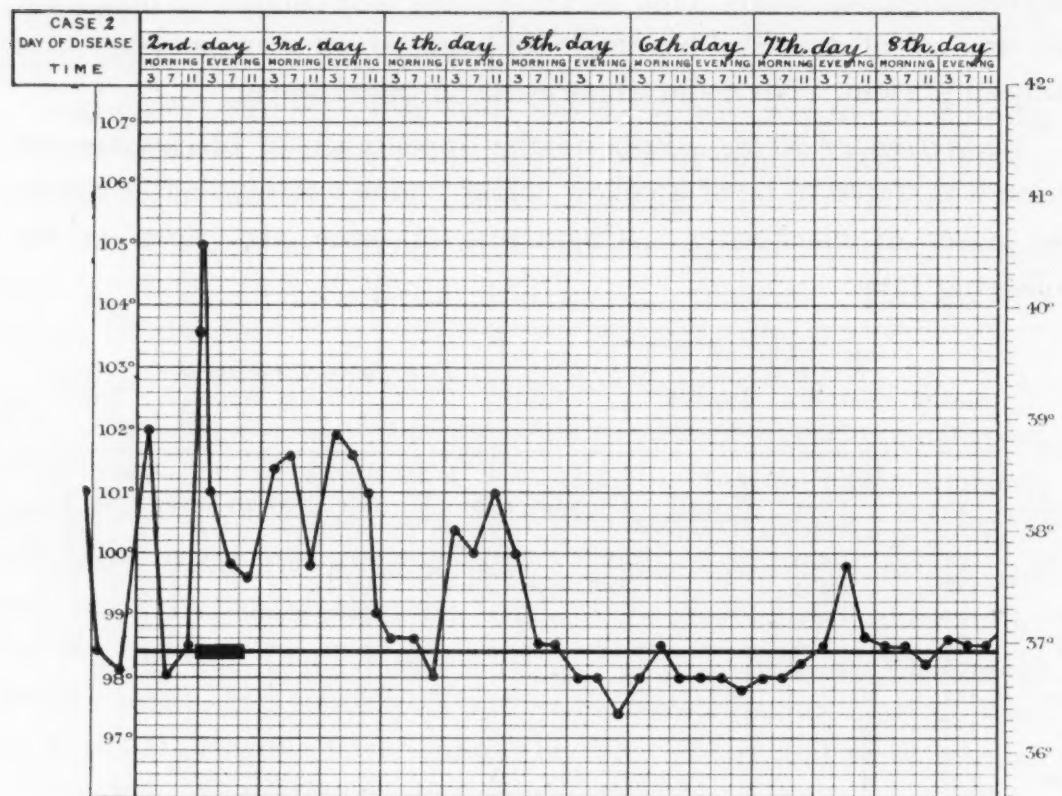


FIG. 71. Blackwater Fever, Case 2. Temperature Chart.

In the succeeding four specimens of urine the deposit consisted chiefly of pus cells, some of which contained red cells or brown pigment; also in smaller amount red cells, partly or wholly decolourised, and granular brown pigment, the latter preponderating over the former; brown granular pigment casts, occasionally hyaline casts; squamous epithelial cells; cylindrical epithelial cells; and bacteria, present in abundance in the urine immediately after being passed. The supernatant liquid was free from odour of decomposition, was turbid, of a dark amber colour, neutral in reaction, and gave on acidifying and boiling one-fifteenth column of coagulated proteid of a light brown colour; on adding tincture of



guaiacum and hydrogen peroxide a faint blue colour was obtained; no bands of haemoglobin or urobilin were seen on spectroscopic examination.

In the remaining specimens of urine the deposit consisted almost entirely of pus cells, sometimes in shreddy masses. In addition granular casts, epithelial cells and abundant bacteria were present.

A week after recovery the urine still contained pus cells and bacilli.

Owing to the absence of oxyhaemoglobin bands in the centrifuged urine at the time of examination, and the presence of pus, it was impossible to determine the amount of dissolved haemoglobin which may have been originally present in the urine.

### BLACKWATER FEVER. CASE 3.

Male, forty-seven years of age. Engineer. European.

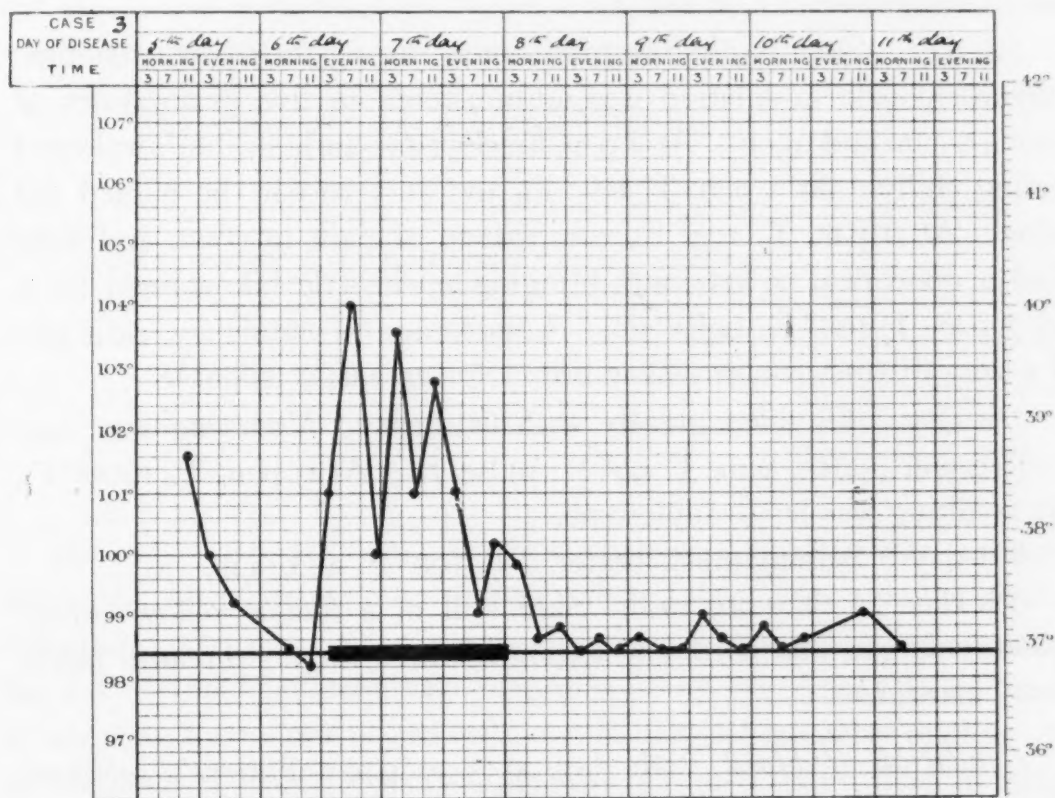


FIG. 72. Blackwater Fever, Case 3. Temperature Chart.

First came to Nyasaland ten years ago. Had fever every two or three months during the first four years of his residence in Nyasaland.

Eight years ago he had his first attack of blackwater fever. This attack was very severe, and he continued to pass blackwater for five days. The next two years he suffered from a good deal of fever, and had three more attacks of blackwater fever. He stated that after each of these attacks he had a relapse. During the next six years he had but little fever, and no further attack of blackwater fever. He used to take about twenty grains of quinine a week during the first five years he spent in Nyasaland, but has taken hardly any for the last five years.

During the last two months he has been living in a very wet camp, but remained comparatively well until about a week ago, when he had a severe attack of fever. This passed off, but was followed by another two days later, and by still another on the next day. He took five grains of quinine on the third day of his illness and ten grains on the fourth day, previously not having taken any for over a year. On the fifth day he was admitted to hospital, and given five grains of quinine. T. 101.6° F.

6th day. Slept badly, perspired freely during the night. Quinine bihydrochloride in solution was administered in five grain doses at 7 a.m., 9 a.m. and noon. At 2 p.m. he slept for an hour, but awakened feeling chilly. At 3 p.m. T. 101° F., and at 3-30 p.m. he passed red urine. At 6 p.m. T. 104° F., complained of pain in chest and legs. Had a good deal of retching, bringing up a little bile-stained fluid. At 7 p.m. T. 100° F., pulse 104. Complained of flatulency, skin was of a very distinct lemon yellow tint. Spleen easily palpable.

7th day. Troubled greatly with flatulency. Vomiting was very troublesome. At 6 a.m. T. 99° F., pulse 78. At 9 p.m. T. 100.2° F., the vomiting was less troublesome.

8th day. Patient much better. T. 100° F. at 6 a.m.

9th day. Felt quite well. Skin still of a lemon yellow colour, spleen enlarged and hard, and extended three fingers' breadth below costal margin.

*Condition of blood.*—5th day, at 3-30 p.m. Patient's oxalated plasma was of a dark orange colour with no red tint. Oxyhaemoglobin bands were faint, the blood plasma containing 0.16 per cent. of oxyhaemoglobin. No autolysin present. A few malarial parasites, but no pigmented leucocytes, were found in the blood.

6th day. At 6 p.m. patient's oxalated plasma was of a dark orange colour with a distinct reddish tint. Oxyhaemoglobin bands were distinct, the blood plasma containing 0.57 per cent. of oxyhaemoglobin. No autolysin present. No malarial parasites or pigmented leucocytes were found in the blood. At 10 p.m. patient's oxalated plasma was of a dark orange colour with a distinct reddish tint. Oxyhaemoglobin bands were distinct, the blood plasma containing 0.4 per cent. of oxyhaemoglobin. No autolysin present in the plasma. No malarial parasites or pigmented leucocytes were found in the blood.

7th day. At 11.45 a.m. patient's oxalated plasma was light yellow coloured, with no reddish tint. Oxyhaemoglobin bands were faint, the blood plasma containing 0.8 per cent. of oxyhaemoglobin. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood.

The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 of % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.4 c.c.	2.0 c.c.	0.1 c.c.	Complete in $\frac{1}{2}$ hour
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete in 1 hour
3	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete in $1\frac{1}{2}$ hours
4	0.8 c.c.	4.6 c.c.	0.1 c.c.	Complete in $2\frac{1}{2}$ hours

8th day. At 10 a.m. patient's oxalated plasma was orange coloured. Oxyhaemoglobin bands were faint, the blood plasma containing 0.08 per cent. of oxyhaemoglobin. On examination with the aid of the haemocrit, the patient's blood was found to contain 20 per cent. by volume of red cells. A haemoglobinometer reading of 30 divisions of Von Fleischl's scale was obtained (equivalent to 0.09 per cent. of wet red cells). A haemocytometer enumeration gave the following figures:—Red corpuscles, 2,612,500 per mm.<sup>3</sup>; leucocytes, 5,470 per mm.<sup>3</sup> Red cell index = 0.58. No autolysin present. No parasites and no pigmented leucocytes were seen in the blood.

The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.4 c.c.	2.0 c.c.	0.1 c.c.	Complete at end of ½ hour
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
3	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 1½ hours
4	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 1½ hours
5	1.05 c.c.	4.6 c.c.	0.1 c.c.	Almost complete at end of 3 hours
6	1.3 c.c.	5.6 c.c.	0.1 c.c.	Trace at end of 3 hours
7	1.5 c.c.	6.4 c.c.	0.1 c.c.	Unaffected at end of 3 hours
8	1.7 c.c.	7.2 c.c.	0.1 c.c.	Unaffected at end of 3 hours

15th day. At 10 a.m. patient's oxalated plasma was light yellow coloured. Oxyhaemoglobin bands were faint, the plasma containing less than 0.11 per cent. of oxyhaemoglobin. No autolysin was found in the plasma, and no malarial parasites or pigmented leucocytes in the blood.

The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.4 c.c.	2.0 c.c.	0.1 c.c.	Complete at end of 3 hours
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 3 hours
3	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 3 hours
4	0.8 c.c.	3.6 c.c.	0.1 c.c.	Trace at end of 3 hours
5	1.05 c.c.	4.6 c.c.	0.1 c.c.	Unaffected at end of 3 hours
6	1.3 c.c.	5.6 c.c.	0.1 c.c.	Unaffected at end of 3 hours
7	1.5 c.c.	6.4 c.c.	0.1 c.c.	Unaffected at end of 3 hours



Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin passed.
5th day 6th day, 3.30 p.m.	— 62 c.c.	— 1.013	— Alkaline	Amber coloured Dark red	Slight whitish deposit consisting of colourless granular matter and granular casts. No red cells.	Oxyhaemoglobin bands present, but no methaemoglobin bands visible.	1.4 % (corresponding to 0.9 gr. of haemoglobin).
" 5.15 "	86 c.c.	1.010	Neutral	"	"	"	1.2 % (corresponding to 1 gr. of haemoglobin).
" 6.0 "	86 c.c.	1.011	Alkaline	"	"	"	1.2 % (corresponding to 1 gr. of haemoglobin).
" 8.0 "	110 c.c.	1.012	"	"	As above, but the deposit is slightly larger in amount.	"	1.3 % (corresponding to 1.2 gr. of haemoglobin).
7th day, 2.0 a.m.	105 c.c.	1.023	"	Dark reddish brown	Small amount of deposit consisting of granular debris and casts with crystals of triple phosphates.	"	Total haemoglobin passed during the day equals 4.2 gr.
" 4.30 "	165 c.c.	1.019	"	Porter coloured	"	"	1.3 % (corresponding to 2.3 gr. of haemoglobin).
" 7.45 "	125 c.c.	1.017	Neutral	"	Small amount of deposit consisting of granular casts with apparently every transition to very granular epithelial casts. Also a few very granular single renal cells mostly columnar, a few cubical. A small amount of free granules about $2\mu$ in diameter. No bacteria; about 10 red cells seen, mostly completely decolourised. Occasionally a pigmented mass.	"	0.73 % (corresponding to 1.2 gr. of haemoglobin).
" 10.30 "	82 c.c.	1.018	Alkaline	"	"	"	0.73 % (corresponding to 0.8 gr. of haemoglobin).
" 2.15 p.m.	210 c.c.	1.013	"	Brown	Slight deposit on centrifugalising, showing same characters as above.	"	0.1 % (corresponding to 0.2 gr. of haemoglobin).
" 6.30 "	203 c.c.	1.014	Neutral	Light red	Slight whitish deposit.	"	0.1 % (corresponding to 0.2 gr. of haemoglobin).
" 9.30 "	135 c.c.	1.015	"	Porter coloured	About $\frac{1}{20}$ th column of whitish deposit.	"	1 % (corresponding to 1.4 gr. of haemoglobin).
8th day, 2.0 a.m.	140 c.c.	1.015	Acid	Brown	Loose whitish deposit, about $\frac{1}{4}$ column, consisting of granular casts and masses, also epithelial casts and cells as previously. Some reddish brown coloration of the casts and masses.	"	Total haemoglobin passed during the day equalled 7.5 gr.
" 7.0 "	148 c.c.	1.014	"	Light brown	Loose whitish deposit of about $\frac{1}{3}$ column.	No bands.	0.1 % (corresponding to 0.2 gr. of haemoglobin).
" 10.30 "	190 c.c.	1.012	"	Brown	Whitish deposit about $\frac{1}{4}$ column.	"	
" till midnight	816 c.c.	1.016	"	Amber coloured	Very slight white deposit.	"	
9th day	1480 c.c.	1.016	"	"	"	"	

The subsequent specimens of the urine were amber coloured, contained no deposit, and gave no precipitate on acidifying with acetic acid and boiling.

#### BLACKWATER FEVER. CASE 4.

Male, about thirty-five years of age. Clerk. Indian.

Has lived in Nyasaland for the last seven years, and has had a good deal of malaria during this time. About ten months ago he had an attack of blackwater fever, since which, except for occasional severe headaches, he has been perfectly well. He has hardly taken any quinine since this attack of blackwater fever.

On the first day of the present illness he had a very severe headache, and felt feverish about mid-day to-day. At 3 p.m. T.  $103^{\circ}$  F. He took ten grains of quinine. At 7 p.m. he took five grains of quinine. At 9 p.m. he had a slight rigor. During the night he states that he had great frequency of micturition, passing water about twenty-five times in very small quantities.

2nd day. When he got up this morning he found that his urine was of a dark red colour. He passed urine five times to-day, the colour increasing until mid-day, and then gradually decreasing. Vomited once this morning.

3rd day. Headache still troublesome; no vomiting; the urine cleared up this morning.

4th day. Patient better; the urine was amber coloured.

5th day. Patient practically well. T.  $98.2^{\circ}$  F. The urine was amber coloured. Spleen large, reaching one and a half inches below the costal margin.

6th day. Five grains of quinine bihydrochloride was given by the mouth, and three grains subcutaneously. The urine remained amber coloured.

From the 7th to the 13th day the patient remained well, and the urine continued to be amber coloured. Three grains of quinine bihydrochloride were administered thrice daily during this period.

14th day. The patient commenced to feel unwell again last evening. This morning he was worse, and had lumbar pains, which did not descend into the testicles or thighs. He vomited several times. He took one dose of three grains of quinine this morning.

At mid-day he had a slight rigor, and about 2 p.m. he passed Burgundy-red urine. At 3 p.m. T.  $104.2^{\circ}$  F. Spleen enlarged and easily palpable, extending about two fingers' breadth below the costal margin.

15th day. Patient was better to-day, and the urine was amber coloured.

16th day. Patient quite well to-day. His recovery continued uninterruptedly.

*Condition of blood.*—5th day. At 10 a.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0.06 per cent. of oxyhaemoglobin. No methaemoglobin bands were observed. No isolysin and no iso-agglutination of the red cells was present. A haemocytometer enumeration gave the following figures:—Red cells = 4,140,000 per mm.<sup>3</sup> No malarial parasites and no pigmented leucocytes were found in the blood.

6th day. On examination of blood films, no malarial parasites or pigmented leucocytes were seen.

16th day. At 10 a.m. patient's oxalated plasma was of a light yellow colour with no red tint, containing 0.13 per cent. of haemoglobin. No autolysin present.

The following observations were made at  $37^{\circ}$  C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.65 c.c.	3.0 c.c.	0.1 c.c.	Partial at end of 3 hours. Almost complete at end of 3 hours. Trace at end of 3 hours.
2	0.8 c.c.	3.6 c.c.	0.1 c.c.	
3	1.05 c.c.	4.6 c.c.	0.1 c.c.	

*Condition of urine.*—On the 1st day, in the evening the urine was of a dark red colour.

2nd day. The urine was porter coloured all day.

3rd day. The urine was amber coloured.

4th day. The urine was dark amber colour, sp. gr. 1.018, no deposit on centrifugalising, no oxyhaemoglobin bands, and no precipitate on acidifying with acetic acid and boiling.

From the 5th day until the 13th day the urine possessed the same characters as on the 4th day.

14th day. The urine became red again this afternoon about 2 p.m.

15th day. The red colour of the urine continued all day, but began to clear towards evening.

16th day. The urine was amber coloured and slightly alkaline. On centrifugalising there was a slight deposit, consisting almost entirely of granular casts and masses. No red cells nor stromata. A few columnar epithelial cells and triple phosphate crystals. Slight precipitate on acidifying with acetic acid and boiling.

17th day. The urine was amber coloured, and gave no precipitate on acidifying and boiling.

Subsequent specimens of the urine were amber coloured and normal.

#### BLACKWATER FEVER. CASE 5.

Male, thirty-seven years of age. Cook. Goanese.

Has been in Nyasaland for ten months. States that he has not suffered from attacks of malaria. Six months ago had an attack of blackwater fever; was ill for one month, the haemoglobinuria extending over ten days and gradually subsiding, without any relapses occurring. Has not had any other illnesses.

1st day. Illness commenced with rigor and profuse sweating. Anorexia, but no vomiting. Has not had quinine.

2nd day. Illness continues. Took ten grains of quinine.

3rd day. Rigor commencing at 6 a.m., accompanied by severe constitutional disturbance. Passed port-wine-coloured urine. Has lumbar pain (bilateral) not extending into testes or groins. Took ten grains of quinine.

4th day. Urine became lighter in colour, was finally faintly red coloured. Heart and lungs normal. Spleen enlarged, projecting at end of respiration about one inch below costal margin. Splenic



dulness increased. Liver dulness normal; liver not palpable. Is extremely weak. Took ten grains of quinine.

5th day. Urine at first red, became later dark amber coloured with no red tint. Is very anaemic and weak. Pain at epigastrium. Complexion sallow. Quinine discontinued.

Subsequently patient's recovery was uninterrupted. No relapse occurred.

*Condition of blood.*—5th day. Blood smear towards close of haemoglobinuria, at 1 p.m., contained no malarial parasites.

6th day. Examination made at 10 a.m., twelve hours after haemoglobinuria had ceased. Blood plasma of light amber colour; no autolysin present; gives faint oxyhaemoglobin bands, 0.13 per cent. of haemoglobin being present in solution. No malarial parasites present in blood smear. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.2 c.c.	1.2 c.c.	0.1 c.c.	Complete at end of ½ hour
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
3	0.05 c.c.	4.6 c.c.	0.1 c.c.	Partial at end of 3 hours
4	1.7 c.c.	7.2 c.c.	0.1 c.c.	Unaffected at end of 3 hours

*Condition of urine.*—5th day (sample of twenty-four hours' urine). Sp. gr. 1.018; neutral to litmus paper; colour, brownish amber; contains a small amount of brownish deposit; after centrifugalisation the supernatant liquid gave no oxyhaemoglobin bands on spectroscopic examination; on acidifying and boiling, about one-fiftieth column of brownish white precipitate was obtained.

6th day. Characters as above, except that on acidifying and boiling slight turbidity appeared, but no precipitate.

The deposit in the urine consists of hyaline masses of casts filled with refractile granules of a faint brownish colour (Fig. 25, p. 53). Many of the casts or masses contain remains of nuclei, and sometimes vacuoles are also seen. No red cells or stromata. No brown amorphous deposit. No crystals.

*Additional note.*—At the end of two and a half months another attack of blackwater occurred, terminating fatally at the end of three days. Patient was not seen by a medical man, and no further particulars of his illness were obtainable.

#### BLACKWATER FEVER. CASE 6.

Male, about thirty-nine years of age. Locomotive inspector. European.

Has lived in Rhodesia for fourteen years. First came to Nyasaland five months ago. Has had a good deal of malaria, both in Rhodesia and Nyasaland. Three years ago he had an attack of blackwater fever, and a second attack a year later. Never taken quinine unless ill. Four days ago he began to feel ill, but was able to continue at his work until yesterday, when he was decidedly worse. He thought he had malaria, and took three doses of quinine of ten grains each.

1st day. Was very unwell all to-day, and in the evening he noticed that his urine was red. T. 103° F.

2nd day. The urine continued red all day, and the patient's general condition was unchanged. Vomiting very troublesome; spleen enlarged and palpable. T. 98·2° F.

3rd day. Patient was rather better this morning. Urine became clear during the morning. Vomiting was still troublesome. T. 98° F.

4th day. Vomiting ceased, and patient felt much better. Urine amber coloured. T. 98·2° F.

*Condition of blood.*—3rd day. At 4 p.m. the oxalated plasma was found to be of a dark orange colour with no red tint, and gave in a column 18 mm. high faint bands of oxyhaemoglobin, equal to 0·16 per cent. of haemoglobin. No isolysin and no iso-agglutinin were present in the patient's blood plasma. No parasites and no pigmented leucocytes were found in the blood.

*Condition of urine.*—1st day. In the evening the urine was porter coloured.

2nd day. The urine remained porter coloured all day.

3rd day. This morning the urine was light red. Later in the day it cleared up, becoming dark amber coloured, sp. gr. 1.014, acid. There was a slight deposit on centrifugalising, consisting of granular casts, few red cells and epithelial cells. The supernatant fluid, when examined with the spectroscope, gave no bands of oxyhaemoglobin or methaemoglobin. There was a slight whitish precipitate on acidifying with acetic acid and boiling.

4th day. Urine was amber coloured, sp. gr. 1.016, no deposit on centrifugalising, and no precipitate on acidifying and boiling.

#### BLACKWATER FEVER. CASE 6a.

See history of preceding attack (Case 6). Has remained in good health since his last attack of blackwater fever about two months ago, with the exception of one attack of malaria which occurred about three weeks ago. During the past two months he has only taken an occasional ten-grain dose of quinine. Yesterday evening he did not feel very well, and, consequently, took ten grains of quinine and went to bed.

1st day. Patient got up this morning feeling better, and took another ten grains of quinine. Later in the day he began to feel very ill, and had to go to bed again. At 7-30 p.m. T. 103.4° F. At 8 p.m. he passed blackwater. Vomiting very troublesome. At 11 p.m. T. 100.2° F.

2nd day. Condition unchanged to-day. Urine brownish coloured.

3rd day. Patient was rather exhausted. Vomiting troublesome. Skin and conjunctivae of a lemon-yellow colour. It was impossible to satisfactorily palpate the spleen owing to the rigidity with which the abdominal walls were held. Urine of brownish colour. T. 98.2° F.

4th day. Patient rather better, but vomiting was still severe. The urine had almost completely cleared during the morning.

5th day. The vomiting had stopped, and the urine was amber coloured.

*Condition of blood.*—3rd day. At 4-30 p.m. patient's oxalated plasma was of a pale orange colour with no red tint, containing 0.16 per cent. of haemoglobin. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood.

*Condition of urine*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin passed.
1st day (evening)	—	—	—	Porter coloured	—	—	—
2nd day	170 c.c.	1'015	Acid	Brown	Deposit on centrifugalising small in amount, consisting of granular casts and debris, red cells and epithelial cells. Also a few crystals of triple phosphates. (There were about 50 red cells to the field).	No bands in the supernatant fluid after centrifugalising	Light brownish precipitate on acidifying and boiling, equalling less than 0.8 % of haemoglobin.
3rd day up till 4.30 p.m.	57 c.c.	1'015	"	"	"	"	Slight precipitate on acidifying and boiling.
4th day, 8.0 a.m.	28.5 c.c.	1'015.	"	"	"	"	"
" 9.0 "	228 c.c.	1'015	"	"	"	"	"
" 9.0 "	28 c.c.	1'015	"	"	"	"	"
5th day	—	1'017	"	Amber coloured	Very slight deposit as above.	"	Faint cloud on acidifying and boiling.

Subsequently the urine was amber coloured and normal.



## BLACKWATER FEVER. CASE 7.

Male, thirty-two years of age. Clerk. Eurasian.

Has been in Nyasaland for six years. One month after coming into the country had an attack of malaria lasting ten days. Nine months later had blackwater fever. Suffered from malaise for about a fortnight, gradually getting worse. Then observed that his urine was of a reddish-black colour, resembling stout. Vomited severely, and had general aching pains. Complexion became sallow, was very weak. At end of three days urine became normal. Patient was well again at end of ten days. Two and a half years ago had dysentery. Six months ago had malaria, his temperature at the time reaching  $105^{\circ}\text{F}$ .

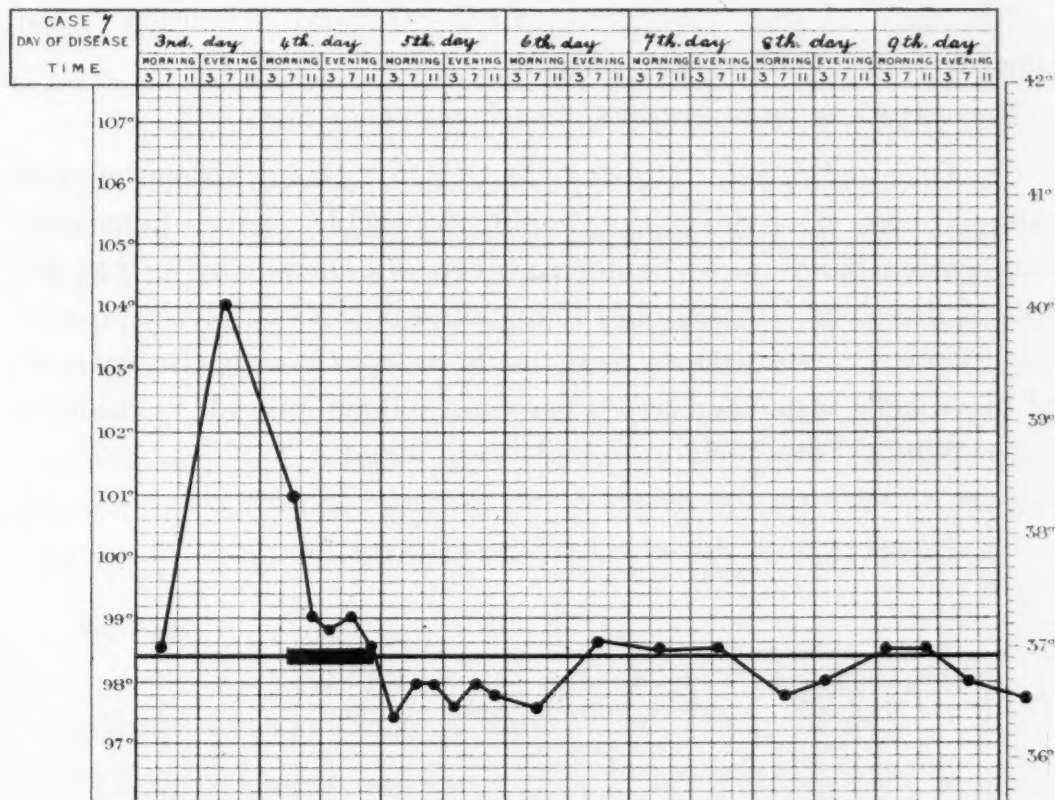


FIG. 73. Blackwater Fever, Case 7. Temperature Chart.

1st day of illness. Felt ill, and went to bed in the afternoon.

2nd day. Did not feel very well in the morning, but did not leave off work. At four o'clock in the afternoon had an attack of ague and went to bed again, shivering and sweating very much. Took ten grains of quinine in five-grain doses.

3rd day. In the morning patient's temperature was not raised. Felt fairly well, except for an empty feeling at the stomach and a sense of weakness. In the afternoon vomited several times, and in the evening had a temperature of  $104^{\circ}$  F. Noticed that his urine was somewhat high coloured, but this he did not think unusual. Took fifteen grains of quinine, in five-grain doses, during the day.

4th day. Noticed that his colour was sallow, and on passing his urine observed that it resembled stout, being dark coloured. Has pain in the hypogastric region. Has had pain in loins for last three days, but no general pains, as was the case during the previous attack of blackwater fever. No constitutional disturbance. Temperature normal. The viscera appear healthy. No recognisable enlargement of the spleen. Is anaemic and sallow.

5th day. Urine amber coloured. General condition good. Appetite fair. Temperature normal.

Recovery was uninterrupted, no relapse occurring.

*Condition of blood.* 4th day, 12-30 p.m. Blood plasma of dark orange colour, no reddish tint being observable; gives faint oxy-haemoglobin bands in a column eighteen millimetres high, 0.13 per cent. of dissolved haemoglobin being present; no autolysin present. The following observations were made at  $37^{\circ}$  C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells.	0.9 % solution of sodium chloride.	1.92 % solution of quinine bihydrochloride.	
1	0.2 c.c.	1.2 c.c.	0.1 c.c.	Complete at end of 35 mins.
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of $1\frac{1}{2}$ hours.
3	1.05 c.c.	4.6 c.c.	0.1 c.c.	Partial at end of 3 hours.
4	1.7 c.c.	7.2 c.c.	0.1 c.c.	Unaffected at end of 3 hours.

7th day. Blood smear negative.

8th day. Blood plasma of light orange colour, contains about 0.06 per cent. of dissolved haemoglobin.

*Condition of urine.*—The characters of the urine are given in the following table :—

Date.	Amount.	Specific gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination.
4th day 9.30 a.m.	650 c.c.	1.030	Slightly alkaline	Porter coloured	$\frac{1}{6}$ col. dark brown	No oxyhaemoglobin bands
" 4.15 "	190 "	—	Alkaline	"	$\frac{1}{10}$ col. dark brown	" "
" 9.30 p.m.	140 "	—	"	Brown amber	Less than above	" "
5th day, 1.40 a.m.	200 "	1.017	Acid	Dark amber	Very slight	
" 7.20 "	150 "	1.027	"	Amber	"	
" 1.30 p.m.	190 "	1.015	"	Light amber	"	
" 6.0 "	130 "	1.010	"	"	"	

The deposit present in the urine on the fourth day consisted of granular masses and casts, the granules being fine, apparently refractile, and brown in colour, held together by hyaline material. A few renal epithelial cells were seen. No red blood cells or stromata. No crystalline deposit.

On centrifugalising the first specimen of urine the supernatant liquid was found to be quite clear, of a dark brown colour without any reddish tint, and on acidifying and boiling gave about one-twentieth column of a light brown flocculent precipitate. The amount of dissolved haemoglobin originally present was therefore small, presumably not exceeding 0.15 per cent. The total amount of haemoglobin in the urine apparently did not exceed that contained in  $980 \times 0.0015 = 1.5$  c.cm. of healthy red cells.

#### BLACKWATER FEVER. CASE 7a.

Male, thirty-two years of age. Clerk. Eurasian.

(For account of previous attack of blackwater fever, two months ago, see preceding report.)

Was well up to ten days ago, when he had febrile disturbance, attributed to malaria, and commenced taking ten grains of quinine every day. As his temperature did not become normal he increased the amount, five days ago, to fifteen grains a day. His temperature then rose to  $104^{\circ}$  F., the rise being accompanied by a rigor and cramp of the stomach, and followed by profuse sweating. Patient expected



that this would be the forerunner of an attack of blackwater, but his urine remained clear and amber coloured. Four days ago, and again three days ago, fifteen grains of quinine, in two and a half grain doses, were taken. His temperature, however, still remained persistently high. Two days ago he took twelve and a half grains of quinine; yesterday he took fifteen grains. During the last three days drank little fluid and passed little urine.

1st day. During the night patient's temperature rose to 105° F. At 5 a.m. passed 350 c.cm. of dark red urine. Has considerable constitutional disturbance. Skin and conjunctivae of a yellow colour. Sweating profusely. Spleen enlarged, projecting about one and a quarter inches below the costal margin at the end of inspiration. From this day onwards no more quinine was taken by patient.

2nd day. Temperature remains low. General condition good. Somewhat depressed. Vomiting of green and yellow bile-stained mucus, troublesome. Pulse good, feels fairly comfortable. Skin still fairly yellow. Urine amber coloured; total amount 18 c.cm.

3rd day. Vomiting continues, otherwise general condition good. Pulse not feeble. Began to perspire when a hot pack was applied, his temperature rising to 102°2F. Total urine 21 c.cm. Received intravenously at 4 p.m. one litre of 0.9 per cent. solution of sodium chloride. Slight rigor one hour later. General condition showed considerable improvement after saline injection. Vomiting ceased. Retained two pints of fluid drink.

4th day. General condition good. Received subcutaneously 800 c.cm. of saline solution. Felt much better after injection. Total urine 11 c.cm.

5th day. Had a good night. General condition remarkably good. Vomited greenish bile on one occasion only. Takes a little semi-fluid food. Total urine 17 c.cm.

6th day. General condition good. Slight vomiting once to-day. Takes and retains a little food. Had slight dyspnoea twice yesterday. Total urine 35 c.cm.



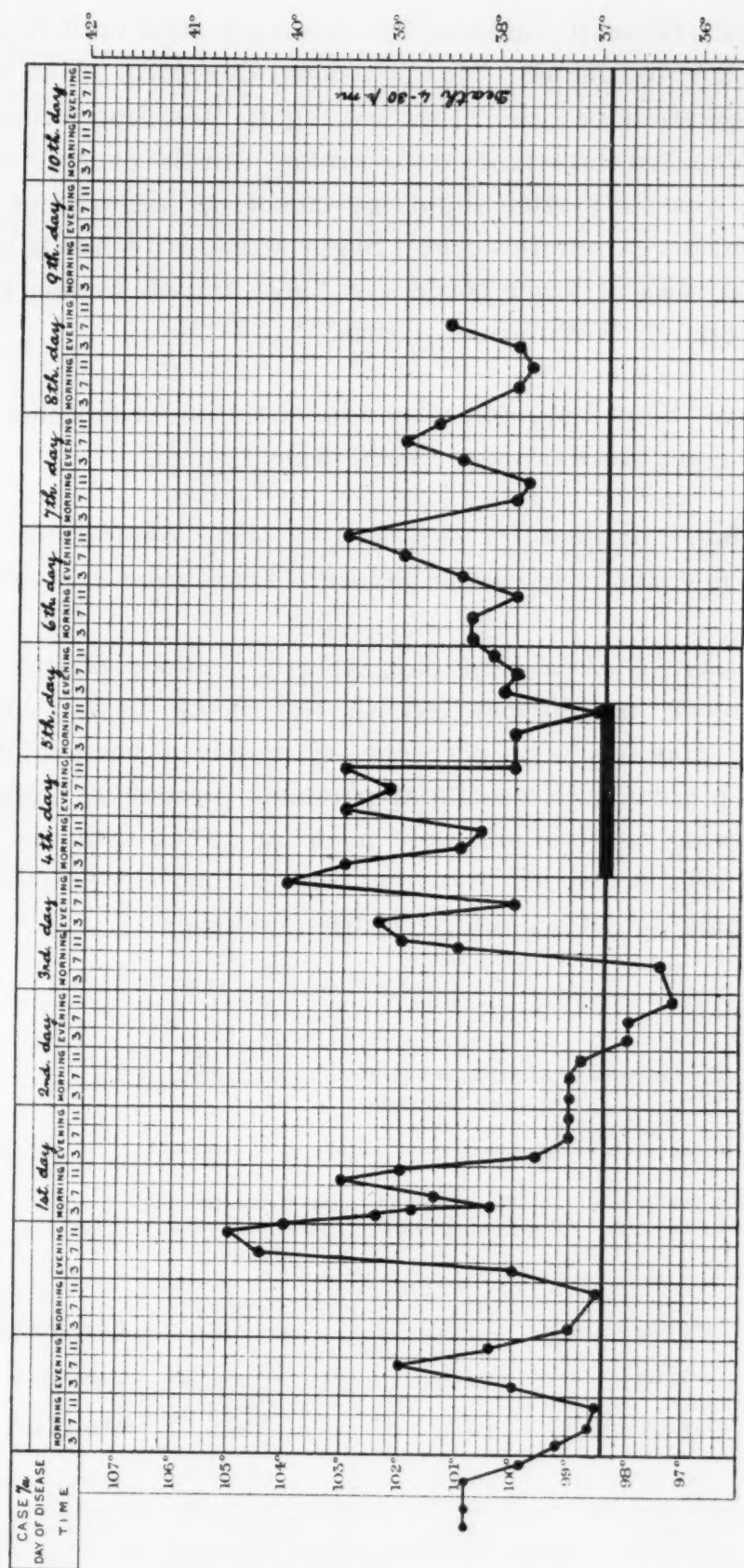


FIG. 74. Blackwater Fever, Case 7a. Temperature Chart.

8th day. General condition has remained good until to-day. Is now lethargic and feeble. Has occasional dyspnoea. Had Cheyne-Stokes respiration for a short time last night. Vomiting recommenced to-day. Vomited three times this morning. In the evening dyspnoea increased, especially after slight movement in bed, patient became restless and his extremities cold. Slight anasarca of hands and face. A few fine râles at lower borders of lungs, breath sounds loud and harsh generally. Total urine 41 c.cm.

9th day. Slept last night after administration of morphia. Much improved again this morning. Total urine 23 c.cm.

10th day. Except for dyspnoea is fairly comfortable. Died suddenly at 5 p.m. from cardiac failure. Total urine 43 c.cm.

*Condition of blood.*—1st day, 10 a.m. Blood plasma of deep orange colour, no distinct reddish tint being recognisable with certainty; contains 0.74 per cent. of dissolved haemoglobin; no autolysin present. No malarial parasites present in blood smear. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete in 1 hour.
2	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete in 2 hours.
3	0.8 c.c.	3.6 c.c.	0.1 c.c.	Marked in 3 hours.
4	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace in 3 hours.
5	1.3 c.c.	5.6 c.c.	0.1 c.c.	Trace in 3 hours.

*Condition of urine.*—The characters of the urine are given in the following table :

Date.	Amount.	Specific gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination.
1st day, 5.0 a.m.	350 c.c.	1.032	Alkaline	Dark porter coloured	$\frac{1}{30}$ col. brown	Oxyhaemoglobin, no met-haemoglobin
" 2.0 p.m.	45 "	1.028	Neutral	"	$\frac{1}{15}$ col. brownish black	Faint O <sub>2</sub> Hb. bands, no methaemoglobin bands
" 6.30 "	30 "	1.020	"	"	$\frac{1}{15}$ "	Same as preceding
" 10.0 "	12 "	1.022	Faintly alkaline	Dark red black	$\frac{1}{30}$ col. "	Strong O <sub>2</sub> Hb. and met-haemoglobin bands
2nd day, 9.30 a.m.	15 "	1.010	Alkaline	Port-wine coloured	$\frac{1}{30}$ " "	Oxyhaemoglobin, no met-haemoglobin
" 1.0 p.m.	1.5 "	—	"	Brown	Very Slight	No haemoglobin
" 2.0 "	1.5 "	—	"	Yellow	"	"
3rd day, 12.30 a.m.	3 "	—	"	"	"	"
" 3.15 p.m.	15 "	—	"	Brownish amber	"	"
" 7.0 "	3 "	—	"	"	"	"
4th day, 4.0 a.m.	2.5 "	—	"	Light yellow	"	"
" 10.0 "	4 "	—	"	"	"	"
" 7.30 p.m.	4.5 "	—	"	"	"	"
5th day, 7.0 a.m.	6 "	1.012	"	Clear yellow	"	"
" 11.30 a.m.	5 "	—	"	"	"	"
" 3.0 p.m.	3 "	—	"	"	"	"
" 6.30 "	3 "	—	"	"	"	"
6th day, 8.0 a.m.	18 "	1.010	"	"	"	"
" 12.30 "	6 "	—	"	"	"	"
" 5.30 "	7 "	—	"	"	"	"
" 7.30 "	2 "	—	Faintly alkaline	"	"	"
7th day, 2.0 a.m.	15 "	1.015	Alkaline	Light yellow	"	"
" 5.0 a.m.	6 "	"	"	"	"	"
" 11.30 "	8 "	"	Faintly alkaline	"	"	"
" 3.30 p.m.	8 "	"	Alkaline	"	"	"
" 9.30 "	8 "	"	"	"	"	"
" 11.5 "	3 "	—	"	"	"	"
8th day, 4.0 a.m.	12 "	1.014	"	"	"	"
" 12.30 p.m.	9 "	"	"	"	"	"
" 3.30 "	7 "	—	"	"	"	"
" 8.30 "	7 "	1.015	"	"	"	"
" 10.0 "	6 "	—	"	"	"	"
9th day, 2.30 p.m. (by catheter)	13 "	1.018	Faintly alkaline	"	"	"
" 5.30 p.m.	3.5 "	1.015	Alkaline	"	"	"
" 7.0 "	7 "	"	"	"	"	"
10th day, 10.0 a.m. (by catheter)	43 "	"	Faintly alkaline	"	"	"

The total amount of urine passed on the first day was 397 c.c. ; on the succeeding nine days the average daily amount was 28.5 c.c.

The deposit in the urine on the first day consisted of yellowish brown granules, forming a precipitate, or arranged in masses or in well defined casts (Fig. 29, p. 100); the individual granules reaching up to  $4.5\mu$  in diameter; unaltered red cells in small numbers (not present in the first specimen of urine); squamous epithelial cells; and (in the first specimen only) triple phosphate crystals. Subsequently the amount of deposit became very small and consisted, even in the last specimen, of red cells; coarse granular casts surrounded by renal tubule cells (Fig. 32, p. 104); granular debris; and renal cells, isolated or in groups.

The first, third, fourth and fifth specimens of urine gave (after centrifugalisation) the following percentages of haemoglobin, determined by means of a comparison spectroscope: 0.41 per cent.; 1.1 per cent.; 1.2 per cent.; 0.8 per cent. These estimations of the oxyhaemoglobin actually present do not represent the amounts actually passing into the urine in the kidney, since much of the haemoglobin had been decomposed. On comparing the chocolate brown precipitate obtained with that yielded by boiling a mixture of blood and urine, it was found that the samples of urine were matched as follows:—1st, with 1.8 per cent. of haemoglobin; 3rd, with 2.5 per cent. of haemoglobin; 4th, with 3.5 per cent. of haemoglobin; 5th, with 3.5 per cent. of haemoglobin. The total haemoglobin lost by the kidneys on the first day did not therefore exceed  $6.4 + 0.99 + 0.75 + 0.42 + 0.52 = 9.06$  g. of wet red blood cells. The yellow coloured urine subsequently passed contained  $\frac{1}{4}$  to  $\frac{2}{3}$  column of coagulable proteid of a slightly brownish white colour.

*Post-mortem examination.*—Body well nourished. Skin no longer sallow. Subcutaneous tissue oedematous generally.

Peritoneal cavity contained about 900 c.cm. of clear yellowish fluid. Spleen much enlarged: 6in. long, 4in. broad, and 2in. thick. Spleen smear showed phagocytosis of red blood cells, but no malarial parasites or pigment. Liver somewhat enlarged, milky opacity on surface, some congestion. Kidneys markedly enlarged, measuring slightly more than 5in. in length, 3in. in breadth, and  $1\frac{1}{4}$ in. in thickness; capsule stripped readily; cortex not markedly congested,



brown in colour; pyramids swollen, dark almost black, with a striated aspect; mucous membrane of pelvis showed punctate ecchymoses; superficial ecchymosis on surface of left kidney, about half an inch in diameter. Intestines distended with gas. Stomach empty; mucous membrane congested.

Recent pleural adhesions on right side; older adhesions on left side; pleural cavities obliterated. Lungs emphysematous; scars on surface of upper lobe near apex; numerous calcified and caseous nodules. Bronchial glands enlarged, not tubercular. Pericardium contained about 10 c.cm. of clear yellowish fluid; valves of heart healthy; myocardium somewhat pale; cavities of heart small.

Bladder normal in aspect. Suprarenals unaltered. Pancreas natural in appearance.

*Microscopical examination.*—The kidneys presented marked distension of the tubules and malpighian capsules. In some of the tubules solid material consisting of coarse brown granules, rarely exceeding  $5\mu$  in diameter, together with irregular masses formed by the aggregation of these granules, and sometimes also free epithelial cells and finely granular or flocculent material, were found partly filling the lumen of the tubule; further details are given on pp. 107-118. The interstitial tissue was free from haemorrhage and showed no cell infiltration or increase of connective tissue. The blood vessels contained no malarial pigment.

The liver showed slight fatty change and some congestion, but no cirrhosis. There was no pigment in the liver cells and no malarial pigment in the blood vessels of the liver.

The spleen exhibited no malarial pigment.

#### BLACKWATER FEVER. CASE 8.

Male, about thirty-eight years of age. Joiner. Chinaman.

Has lived in Delagoa Bay and Chinde for ten years. First came to Nyasaland two years ago. During this time he has had a consider-

able amount of fever, but has never previously had blackwater fever. Has rarely taken quinine except after an attack of fever.

He commenced to be ill with fever three days ago. Stayed in bed and took fifteen grains of quinine on this and on each of the two following days.

4th day. At 2 p.m. he had a rigor, T.  $104.4^{\circ}$  F., and shortly afterwards passed porter coloured urine. Had slight vomiting.

5th day. Patient felt better this morning. The vomiting had ceased, and the urine during the morning became almost clear. About noon the urine became red again. At 2-30 p.m. T.  $102^{\circ}$  F. Vomiting was troublesome. The spleen was enlarged and easily palpable.

6th day. Patient better to-day. Urine amber coloured. At 10 a.m. three grains of quinine bihydrochloride were given hypodermically, and at 2 p.m. two and a half grains were given by the mouth.

7th day. About noon to-day there was a slight relapse, the urine becoming claret coloured. One grain of quinine was given three times to-day.

8th day to 13th day. Quinine bihydrochloride was administered thrice daily in two grain doses. The urine remained amber coloured during this period.

14th day. Three grains of quinine bihydrochloride were given thrice daily.

15th day. Patient remained well, no further relapse; the urine continued to be amber coloured.

*Condition of blood.*—5th day. At 1-45 p.m. patient's oxalated plasma was of a deep orange colour, with no red tint, containing 0.13 per cent. of haemoglobin. No bands of methaemoglobin were seen on examining a column of the plasma 18 mm. deep. No

autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added :—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.4 c.c.	2.0 c.c.	0.1 c.c.	Complete at end of 3 hours
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 3 hours
3	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 3 hours
4	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 3 hours
5	1.05 c.c.	4.6 c.c.	0.1 c.c.	Almost complete at end of 3 hours
6	1.3 c.c.	5.6 c.c.	0.1 c.c.	Slight at end of 3 hours
7	1.5 c.c.	6.4 c.c.	0.1 c.c.	Slight at end of 3 hours
8	1.7 c.c.	7.2 c.c.	0.1 c.c.	Trace at end of 3 hours

6th day. At 10 a.m. patient's oxalated plasma was of a deep orange colour, with no red tint, containing 0.07 per cent. of haemoglobin in solution. No parasites or pigmented leucocytes were found in blood smears. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added :—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 3 hours
2	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 3 hours
3	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 3 hours
4	1.05 c.c.	4.6 c.c.	0.1 c.c.	Slight at end of 3 hours
5	1.3 c.c.	5.6 c.c.	0.1 c.c.	Trace at end of 3 hours
6	1.5 c.c.	6.4 c.c.	0.1 c.c.	Nil at end of 3 hours

*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day	—	—	—	Porter coloured	—	—	—
5th day, noon	—	—	Acid	Port wine coloured	Deposit on centrifugalising slight, consisting of granular casts and few renal epithelial and red cells	Oxyhaemoglobin and methaemoglobin bands, both distinct	0.2 %
" 1.45 p.m.	—	—	"	"	"	"	0.1 %
" 2.50 "	114 c.c.	1.012	"	Dark brown	"	"	0.15 % (corresponding to 0.17 gr. of haemoglobin)
" 5.0 "	170 c.c.	1.008	"	"	"	No bands	Trace of precipitate on acidifying and boiling
" 7.0 "	114 c.c.	1.008	"	Dark amber coloured	Very slight deposit	"	"
" 10.0 "	114 c.c.	1.008	"	"	"	"	"
" 11.30 "	170 c.c.	1.014	"	"	"	"	"
6th day, 6.0 a.m.	285 c.c.	1.014	"	"	Very slight deposit as above	"	"
" 9.0 "	114 c.c.	1.020	"	"	"	"	"
" 10.45 "	140 c.c.	1.022	"	"	"	"	"
" 11.30 "	85 c.c.	1.016	"	"	"	"	"
" noon	114 c.c.	1.008	"	Pale amber coloured	"	"	"
" 1.30 p.m.	285 c.c.	1.005	"	"	"	"	No precipitate on acidifying and boiling
7th day, noon	—	—	—	Red	"	"	"

The next day the urine was amber coloured and normal and the subsequent specimens were all normal.



## BLACKWATER FEVER. CASE 9.

Male, thirty-eight years of age. Contractor. European.

Has been in the Tropics for fifteen years. Has had dysentery twice, the last attack two years ago. Has had 'fever' (malaria) many times. Has suffered from rheumatic pains. Has not had blackwater fever before present attack.

Has not been feeling well for some weeks. Appetite has been poor. Has been inclined to doze in daytime. For the last three weeks has had 'fever,' for which he has been taking a Durban patent 'cure.' His urine has been of a dark brownish colour for last six weeks. Yesterday took nine grains of quinine in tabloid form.

1st day. Thinks he passed blackwater in the morning when at stool, but regarded the colour of his urine as due to bile. Took nine grains of quinine during the day. Stayed indoors and had a rigor at mid-day, when he passed more blackwater, which he still regarded as due to bile. At 6 p.m. his urine was porter coloured, about 170 c.cm. being passed. Temperature at this time 104.2 F.

2nd day. Constitutional disturbance continues. Urine still remains porter coloured. Vomited about twenty times during evening and night.

3rd day. Urine still red. Lighter coloured in evening.

4th day. Urine at first red, later became amber coloured.

5th day. During the 2nd, 3rd, 4th and 5th days only about 600 c.c. of urine was passed, together with a small but inconsiderable amount in the stools, and suppression of urine was feared. Cardiac action feeble.

6th day. Passing urine more freely.

7th day. Continues to pass urine freely. Is very pale and anaemic, but not wasted. Not sallow. Pulse hard and sharp, of high tension. Cardiac dulness abolished. Apex beat cannot be felt, but the first sound is at a maximum three and a half inches from midsternal line and about half an inch inside the nipple line. No adventitious

sounds. Second sound at base over aortic orifice accentuated. Liver not palpable. Liver dullness not increased. Spleen extends at the end of inspiration, about four inches beyond the costal margin. Splenic dullness considerably increased. During the attack of blackwater has had pain in loins, not descending into testes or thighs.

*Condition of blood.*—8th day. On pricking patient's finger the blood obtained was watery in aspect. The red cells were relatively small in amount, and rapidly sank in the (oxalated) plasma. Plasma light yellow, gives a faint oxyhaemoglobin spectrum, but no methaemoglobin bands, in a layer eighteen millimetres high; amount of dissolved haemoglobin is less than 0.06 per cent. On testing for autolysin (eight hours after withdrawal of blood) complete haemolysis was obtained in two and a half hours (Table 29); bacteria were, however, present at the time. (On testing blood plasma for autolysin three and a half months later a negative result was obtained.)

No malarial parasites found in blood smear. The following observations were made at 37° C. with the patient's red blood cells, suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete in 1 hour
2	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete in 2½ hours
3	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace at end of 3 hours

*Condition of urine.*—8th day. Yellow in colour. On adding a drop of acetic acid and boiling becomes cloudy, and an inconsiderable amount (about a  $\frac{1}{40}$  column) of whitish precipitate is formed. Contains a small amount of suspended matter consisting chiefly of pus cells, together with squamous epithelial cells and a few granular masses or casts of a faint brownish tint; no red cells or stromata; no granular pigment.

## BLACKWATER FEVER. CASE 10.

Male, twenty-six years of age. Engineer. European.

Has lived in Nyasaland for two years, and during this time he has had a considerable amount of fever. He has taken very little quinine during the last twelve months. About three weeks ago he had a severe attack of fever which lasted four or five days. A few days later he was run over by a trolley, and has been confined to bed more or less since. He was unable to sleep and felt very ill. Three days ago he felt particularly unwell and had a sore throat. The next day his temperature was  $102^{\circ}$  F., and he took seven and a half grains of quinine. Yesterday his temperature was  $103^{\circ}$  F., and he took another seven and a half grains of quinine.

1st day. He felt better this morning and took ten grains of quinine and was able to get up. At noon he had five grains of quinine and took a short walk. At 4 p.m. he suddenly felt very ill. He had a severe rigor. T.  $105^{\circ}$  F. At 5 p.m. he passed blackwater. Had troublesome vomiting and flatulency all night.

2nd day. Vomiting had ceased and patient felt better. Spleen enlarged and easily palpable, extending one and a half inches below the costal margin.

3rd day. Patient was better to-day; the urine was not so darkly coloured.

4th day. About noon there was a relapse. T.  $103^{\circ}$  F.

5th day. Patient admitted to hospital this evening. Blackwater continued. T.  $103^{\circ}$  F.

6th day. The urine was amber coloured this morning. About noon it became red again, but cleared up in the afternoon. There was another relapse late in the evening.

7th day. The urine remained coloured all day; in the evening T.  $103.6^{\circ}$  F.

8th day. Urine still contained haemoglobin. Quinine bihydrochloride was given in solution in two and a half grain doses at 6 a.m. and 9 a.m. Later in the day the urine became amber coloured. T.  $102.4^{\circ}$  F.



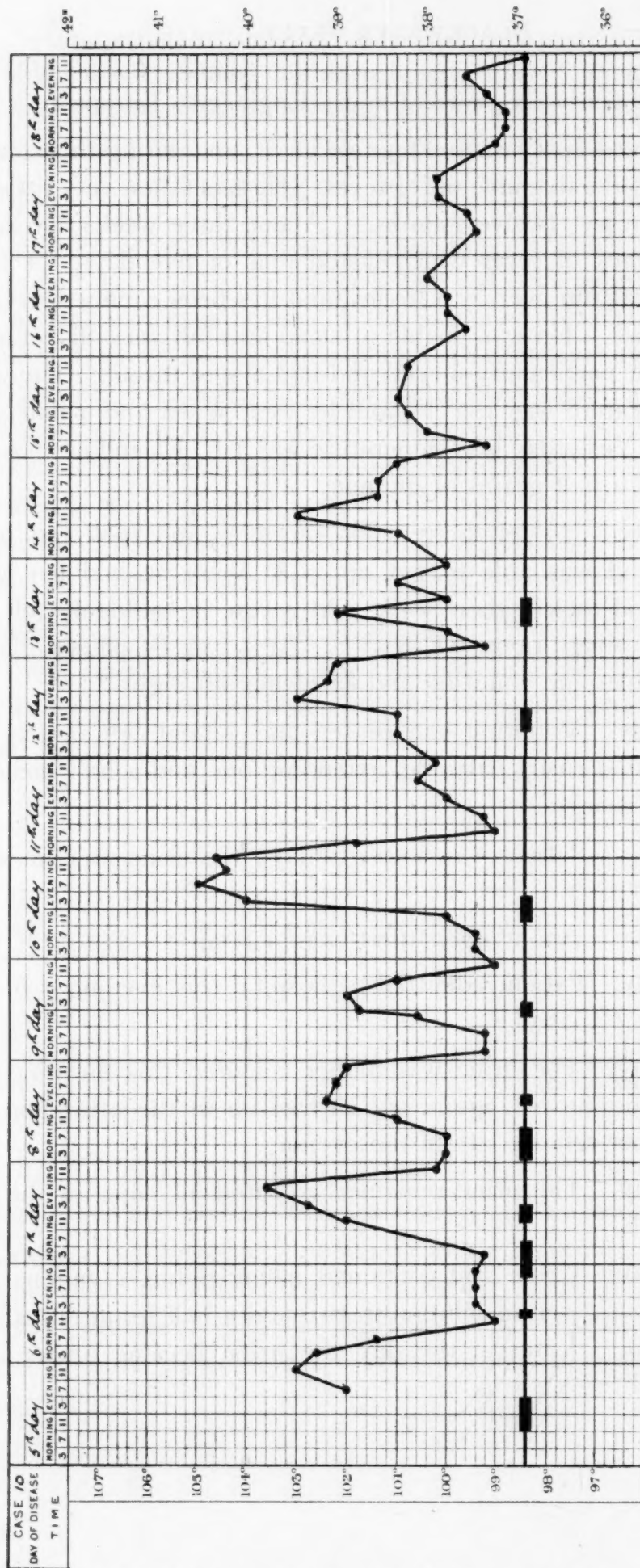


FIG. 75. Blackwater Fever, Case 10. Temperature Chart.



9th day. The urine was coloured again this morning, but cleared up in the evening. Quinine bihydrochloride was given as before in two doses of two and a half grains each. T.  $102^{\circ}$  F.

10th day. The urine, which was clear this morning, became deeply red coloured about mid-day. Quinine bihydrochloride was given in solution in three doses of three and three quarter grains each. T.  $104^{\circ}$  F. Patient appeared to be in fairly good condition.

11th day. The urine remained clear all day. Quinine bihydrochloride was given in three doses of five grains each. T.  $100.6^{\circ}$  F.

12th day. There was another slight relapse this morning. Quinine bihydrochloride was given again in three doses of five grains each. T.  $103^{\circ}$  F.

13th day. The urine, which had become amber coloured yesterday afternoon, was again red about noon to-day. Quinine bihydrochloride was given in three doses of five grains each. T.  $102^{\circ}$  F.

14th day. There was a slight brownish coloration of the urine about noon to-day, but no detectable amount of haemoglobin. No quinine was given to-day. T.  $103^{\circ}$  F.

15th day. Urine normal all day. No quinine given. T.  $101^{\circ}$  F.

16th day. Urine normal. No quinine given. T.  $100.4^{\circ}$  F.

Patient remained well and had no further relapses. About fourteen days later small doses of quinine were commenced and the quantity gradually increased until fifteen grains were given daily. No further relapses occurred

*Condition of blood.*—2nd day. At 11 a.m. patient's oxalated plasma was of a deep orange colour with a distinct reddish tint, and gave marked bands of oxyhaemoglobin in a column 18 mm. high, representing 0.85 per cent. of dissolved haemoglobin. Faint bands of methaemoglobin were visible. No autolysin was present in the serum, and no parasites or pigmented leucocytes were found in the blood. The following observations were made at  $37^{\circ}$  C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.4 c.c.	2.0 c.c.	0.1 c.c.	Complete at end of 1 hour
2	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
3	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 2 hours
4	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 2½ hours
5	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace at end of 3 hours
6	1.3 c.c.	5.6 c.c.	0.1 c.c.	Nil at end of 3 hours

3rd day. At 10-30 a.m. patient's oxalated plasma was deep orange, with no red tint, containing 0.09 per cent. of haemoglobin in solution. The bands of methaemoglobin were visible but faint. No parasites or pigmented leucocytes were found in the blood.

5th day. At 11 a.m. patient's oxalated plasma was a deep orange colour and gave, in a column 18 mm. high, distinct oxyhaemoglobin bands, the amount of dissolved haemoglobin being 0.3 per cent. The methaemoglobin bands were just visible. No autolysin present in the plasma. The appearance of the blood when withdrawn was distinctly watery. No parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
2	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 2 hours
3	0.8 c.c.	3.6 c.c.	0.1 c.c.	Very marked at end of 3 hours
4	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace at end of 3 hours

6th day. At 11 p.m. patient's oxalated plasma was of a dark orange colour with no red tint; it gave distinct oxyhaemoglobin bands, 0.2 per cent. of haemoglobin being present in solution. No parasites or pigmented leucocytes were found in the blood.

*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 6.30 p.m.	12 c.c.	1.022	Acid	Dark red	Considerable amount of deposit consisting entirely of granular casts and debris. No red cells.	Marked haemoglobin and methaemoglobin bands.	0.6% (corresponding to 0.1 gr. of haemoglobin). Total haemoglobin passed during day equals 0.1 gr.
2nd day, 11.0 a.m.	—	—	"	Port wine red	Deposit $\frac{3}{8}$ column consisting of granular casts and squamous epithelium. No red cells.	"	1.1%
" 3.30 p.m.	205 c.c.	1.024	"	Porter coloured	Deposit considerable, consisting of granular casts and debris and epithelial cells. Very few red cells (about 1 in a field).	"	0.65% (corresponding to 1.3 gr. of haemoglobin).
" 4.30 "	130 c.c.	1.020	"	"	"	"	0.85% (corresponding to 1.1 gr. of haemoglobin).
" 6.30 "	95 c.c.	1.020	"	"	"	"	1.1% (corresponding to 1.1 gr. of haemoglobin).
" 8.0 "	200 c.c.	1.016	"	"	"	"	1.1% (corresponding to 2.2 gr. of haemoglobin).
3rd day, 10.30 a.m.	—	—	—	Brown	Slight deposit consisting mainly of granular casts.	Bands of oxyhaemoglobin and methaemoglobin, both faint.	Total amount of haemoglobin passed during day equals 5.7 gr. 0.1%
" 4.0 p.m.	120 c.c.	1.018	Acid	"	"	"	—
" 7.0 p.m.	—	—	—	"	"	"	—
4th day, 10.0 a.m.	—	1.024	Acid	"	Considerable deposit on centrifugalising consisting almost entirely of granular casts; very few red cells.	"	—
" noon	—	1.022	"	Porter coloured	"	Strong bands of oxyhaemoglobin and methaemoglobin.	0.8%
" 2.0 p.m.	—	1.016	"	Port wine colour	"	Bands of oxyhaemoglobin and methaemoglobin just visible.	Large chocolate coloured precipitate on acidifying and boiling equalling $\frac{1}{2}$ column.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day 4.0 p.m.	—	1.012	Acid	Light brown	Slight deposit as above.	Bands of oxyhaemoglobin and methaemoglobin just visible.	Light brown precipitate on acidifying and boiling equalling $\frac{1}{10}$ column.
" 5.30 "	—	1.003	"	Amber	No deposit.	"	Very faint cloud on acidifying and boiling.
5th day, 9.30 a.m.	—	1.020	"	Porter coloured	Deposit slight consisting of granular casts and epithelial cells.	Bands of oxyhaemoglobin and methaemoglobin well marked.	0.75 %; large chocolate coloured precipitate on acidifying and boiling equalling $\frac{1}{2}$ column.
" 11.0 "	—	1.014	"	Dark red	"	"	1.6 %; chocolate coloured precipitate on acidifying and boiling equalling $\frac{1}{3}$ column
" 2.30 p.m.	200 c.c.	1.010	"	Reddish brown	"	Faint bands of oxyhaemoglobin and methaemoglobin.	0.05 %; chocolate coloured precipitate on acidifying and boiling equals $\frac{1}{5}$ col.
" 4.30 "	300 c.c.	1.002	"	Amber	No deposit.	No bands	Slight white precipitate on acidifying and boiling.
" 7.0 "	300 c.c.	1.005	"	"	"	Bands of oxyhaemoglobin just visible.	"
" 9.30 "	200 c.c.	1.010	"	"	"	No bands	"
" 11.30 "	—	1.004	"	"	"	"	"
6th day, 3.0 a.m.	—	1.011	"	"	"	"	No precipitate on acidifying and boiling.
" 6.15 "	—	1.012	"	"	"	"	"
" noon "	—	1.020	"	Brown	Slight deposit consisting of granular casts.	Faint bands of oxyhaemoglobin.	Brownish precipitate on acidifying and boiling equals $\frac{1}{5}$ column.
" 2.30 p.m.	—	1.010	"	Dark amber	"	No bands	Very slight precipitate on acidifying and boiling.
" 10.30 "	—	1.012	Neutral	Light red	Whitish deposit on centrifugalising about $\frac{1}{2}$ column consisting of granular casts and triple phosphate crystals.	Well marked bands of oxyhaemoglobin.	0.36 %; brownish precipitate on acidifying and boiling equals $\frac{1}{5}$ column.
7th day, 6.30 a.m.	224 c.c.	1.020	Alkaline	"	"	"	0.3 % (corresponding to 0.7 gr. of haemoglobin).



Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
7th day 7.30 a.m.	285 c.c.	1.018	Acid	Dark amber	No deposit.	No bands	No precipitate on acidifying and boiling.
" 11.0 "	257 c.c.	1.015	Alkaline	Porter coloured	Slight deposit consisting of granular casts and crystals of triple phosphates.	Methaemoglobin bands stronger than the oxyhaemoglobin bands.	0.82 % (corresponding to 2 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equals $\frac{1}{8}$ column.
" 4.0 p.m.	198 c.c.	1.016	"	Amber	No deposit.	No bands	No precipitate on acidifying and boiling.
" 6.30 "	198 c.c.	1.012	"	"	"	"	"
" 9.0 "	198 c.c.	—	"	"	"	"	"
8th day, 5.30 a.m.	370 c.c.	1.022	Acid	Porter coloured	Brownish deposit about $\frac{1}{10}$ th column, consisting of granular casts.	Well marked bands of oxyhaemoglobin and methaemoglobin.	Total haemoglobin passed during the day equalled 2.6 gr.
" 7.30 "	114 c.c.	1.018	"	"	"	"	0.8 % (corresponding to 1.9 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled $\frac{1}{8}$ column.
" 9.0 "	171 c.c.	1.010	"	Light reddish brown	"	"	0.8 % (corresponding to 0.9 gr. of haemoglobin). Brownish precipitate on acidifying and boiling equalled $\frac{1}{10}$ th column.
" 11.30 "	198 c.c.	1.013	Alkaline	Amber	Very slight deposit.	No bands	Slight turbidity on acidifying and boiling.
" 1.0 p.m.	170 c.c.	1.016	Acid	"	"	"	"
" 3.30 "	86 c.c.	1.015	"	Brownish red	"	"	0.3 %
" 6.0 "	142 c.c.	1.013	"	Amber	"	"	Slight turbidity on acidifying and boiling.
" 10.0 "	198 c.c.	1.015	"	"	"	"	"
9th day, 3.0 a.m.	198 c.c.	1.018	Alkaline	"	"	"	Total haemoglobin passed during day equalled 2.8 gr.
" 5.45 "	114 c.c.	1.018	"	"	"	"	Slight turbidity on acidifying and boiling.
" 7.45 "	142 c.c.	1.018	"	"	"	"	"

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
9th day 10.0 a.m.	142 c.c.	1'014	Acid	Reddish brown	Slight brownish deposit consisting mainly of granular casts.	Oxyhaemoglobin bands but no methaemoglobin bands present.	0.8% (corresponding to 1.1 gr. of haemoglobin). Brownish precipitate on acidifying and boiling equalled $\frac{1}{3}$ column.
" 11.0 "	84 c.c.	1'015	"	"	"	"	1.0% (corresponding to 0.8 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled $\frac{1}{4}$ column.
" 12.30 p.m.	98 c.c.	1'013	"	Amber	No deposit.	No bands.	Whitish precipitate on acidifying and boiling $\frac{1}{12}$ column.
" 1.30 "	57 c.c.	1'013	"	"	"	"	Slight precipitate on acidifying and boiling.
" 5.30 "	170 c.c.	1'011	"	"	"	"	"
" 7.0 "	170 c.c.	1'012	"	"	"	"	"
" 9.30 "	114 c.c.	1'014	"	"	"	"	"
" 10.30 "	85 c.c.	1'014	"	"	"	"	"
10th day, 3.0 a.m.	120 c.c.	1'015	Alkaline	"	"	"	Total haemoglobin passed during the day 1.9 gr.
" 6.0 "	130 c.c.	1'016	"	"	"	"	No precipitate on acidifying and boiling.
" 10.0 "	180 c.c.	1'015	"	"	"	"	"
" 11.30 "	320 c.c.	1'002	"	Light brown	Slight deposit consisting of granular casts.	Faint bands of oxyhaemoglobin.	Light brownish precipitate on acidifying and boiling $\frac{1}{30}$ column.
10th day, 11.50 a.m.	40 c.c.	1'011	Alkaline	Port wine red	"	Strong oxyhaemoglobin and methaemoglobin bands.	1.4% (corresponding to 0.5 gr. of haemoglobin). Dark chocolate coloured precipitate on acidifying and boiling $\frac{1}{4}$ column.
" 12.30 p.m.	140 c.c.	1'012	"	Dark red	"	"	2.1% (corresponding to 2.9 gr. of haemoglobin). Dark chocolate coloured precipitate on boiling $\frac{2}{3}$ column.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
10th day 1.20 p.m.	60 c.c.	1.010	Alkaline	Light red	Slight deposit consisting of granular casts.	Strong oxy-haemoglobin and methaemoglobin bands. No bands.	1% (corresponding to 0.6 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{2}$ column. No precipitate on acidifying and boiling.
" 3.0 "	200 c.c.	1.010	"	Amber	No deposit.	"	"
" 6.0 "	150 c.c.	1.010	"	"	"	"	"
11th day, 10.0 a.m.	600 c.c.	1.011	Acid	"	"	"	Total haemoglobin passed during the day 4 gr. No precipitate on acidifying and boiling.
" 11.30 "	200 c.c.	1.012	"	"	"	"	"
" 2.15 p.m.	180 c.c.	1.012	"	"	"	"	"
" 4.45 "	120 c.c.	1.012	"	"	"	"	"
" 7.0 "	160 c.c.	1.011	"	"	"	"	"
" 11.0 "	200 c.c.	1.013	"	"	"	"	"
12th day, 5.0 a.m.	150 c.c.	1.013	"	Dark amber	Deposit on centrifuging slight, consisting of granular casts and phosphates.	Bands of oxy-haemoglobin distinct.	0.8% (corresponding to 0.9 gr. of haemoglobin). Chocolate precipitate on boiling $\frac{1}{2}$ column.
" 6.30 "	170 c.c.	1.011	Alkaline	Port wine red	"	"	"
" 10.30 "	180 c.c.	1.011	Acid	Deep amber	Slight deposit as above.	No bands.	Very slight precipitate on acidifying and boiling.
" 11.50 "	200 c.c.	1.012	"	Brownish red	"	Bands of oxy-haemoglobin present but no methaemoglobin bands.	0.6% (corresponding to 1.2 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{2}$ column.
" 2.0 p.m.	190 c.c.	1.010	"	Dark amber	No deposit.	No bands.	Very faint cloud on acidifying and boiling.
" 5.30 "	240 c.c.	1.010	Alkaline	"	"	"	"
" 8.0 "	110 c.c.	1.010	Acid	"	"	"	No precipitate on acidifying and boiling.
" 10.0 "	205 c.c.	1.012	"	"	"	"	"
" 11.0 "	195 c.c.	1.012	"	"	"	"	Total haemoglobin passed during day 1.6 gr.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
13th day, 2.0 a.m.	150 c.c.	1.014	Alkaline	Dark amber	No deposit.	No bands.	No precipitate on acidifying and boiling.
" 4.0 "	120 c.c.	1.015	Acid	"	"	"	"
" 8.0 "	150 c.c.	1.014	Alkaline	Brownish red	Slight deposit of granular casts.	Bands of oxyhaemoglobin just visible. Distinct bands of oxyhaemoglobin.	Slight precipitate on acidifying and boiling.
" 10.0 "	70 c.c.	1.015	"	"	"	"	0.6% (corresponding to 0.4 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{4}$ column.
" 2.0 p.m.	160 c.c.	1.014	Acid	Brownish red	"	"	0.6% (corresponding to 1 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{4}$ column.
" 4.30 "	140 c.c.	1.012	"	"	"	"	0.4% (corresponding to 0.5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{4}$ column.
" 7.0 "	240 c.c.	1.012	"	Light brown	"	No bands.	Faint cloud on acidifying and boiling.
" 9.30 "	140 c.c.	1.014	"	"	No deposit.	"	Total haemoglobin passed during day 1.9 gr.
14th day, 1.0 a.m.	160 c.c.	1.014	Alkaline	"	"	"	Faint cloud on acidifying and boiling.
" 6.0 "	180 c.c.	1.015	"	"	"	"	"
" noon "	240 c.c.	1.015	Acid	"	"	"	"
" 5.30 p.m.	240 c.c.	1.015	"	"	"	"	"
" 7.0 "	210 c.c.	1.015	"	Amber	"	"	No precipitate on acidifying and boiling.
" 10.0 "	420 c.c.	1.008	Alkaline	"	"	"	"
15th day	1310 c.c.	1.015	Acid	"	"	"	"
16th day	1210 c.c.	1.014	"	"	"	"	"
17th day	1413 c.c.	1.011	"	"	"	"	"
18th day	1320 c.c.	1.011	"	"	"	"	"
19th day	1230 c.c.	1.012	"	"	"	"	"

Subsequently the urine continued to be normal and there were no further relapses.



## BLACKWATER FEVER. CASE 11.

Male, thirty-eight years of age. Locomotive inspector. European.

First came to Nyasaland nine months ago, and has lived chiefly in Port Herald and the Lower Shiré district. Previous to coming to Nyasaland he had spent five years in Rhodesia. During the last nine months he has had a good deal of malaria but has never previously had blackwater fever. He has very rarely taken any quinine. During the last week he has been very unwell, and two days ago he took ten grains of quinine. The next day he felt no better, but took no more quinine.

1st day of present illness. At 9 a.m. he felt very ill and took ten grains of quinine. At 10-15 a.m. he passed porter-coloured urine and again at 11-30 a.m. T.  $103^{\circ}$  F. During the remainder of the day patient was very restless vomited several times, and passed no more urine.

2nd day. Slept a little during the night and took large quantities of fluid by the mouth. At 10 a.m. he passed a little urine, which was porter coloured. Vomited several times. Skin and conjunctivae of a very distinct lemon yellow tint. Spleen easily palpable. T.  $105^{\circ}$  F. At 5 p.m. T.  $101^{\circ}$  F.

3rd day. Had a very bad night; passed no urine during the night. Continued to take large quantities of fluid by the mouth. At 3-30 p.m. he passed 10 c.cm. of dark red urine. At 6 p.m. the skin was very sallow and the conjunctivae lemon-yellow. T.  $101^{\circ}$  F. Vomiting and hiccough very troublesome.

4th day. Slept very little. T.  $100^{\circ}$  F. Urine amber coloured. The total amount of urine passed during the day was 48 c.cm.

5th day. Condition of patient was unchanged. Urine amber coloured. Temperature normal. The total amount of urine passed during the day was 92 c.cm.

6th day. Total amount of urine passed to-day was 73 c.cm.

7th day. Vomiting and hiccough continue. Total urine passed during day was 93 c.cm.

8th day. Patient felt better, slept well, pulse and temperature normal, and the vomiting was not so troublesome. Amount of urine passed during day was 85 c.cm.

9th day. Patient had a good night and slept well. At 7 a.m. about 8 c.cm. of urine were passed. After this no more urine was voided, and very severe dyspnoea set in about 4 p.m. Finally at 7 p.m. patient died during an attack of dyspnoea.

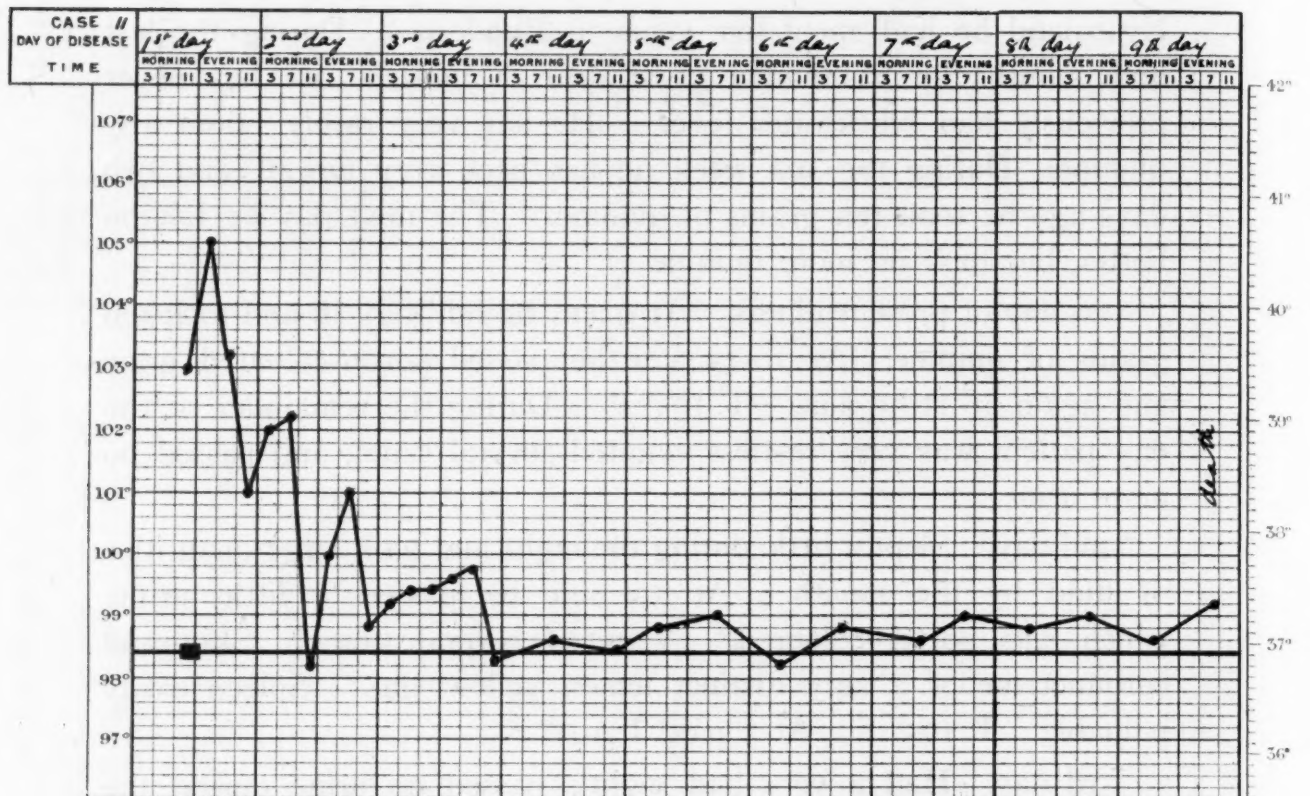


FIG. 76. Blackwater Fever, Case 11. Temperature Chart.

*Condition of blood.*—3rd day. At 7 p.m. patient's oxalated plasma was of a deep orange colour with no reddish tint, and gave marked bands of oxyhaemoglobin in a column 18 mm. high, representing 0.25 per cent. of dissolved haemoglobin. Methaemoglobin bands were

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells.	0.9 % solution of sodium chloride.	1.92 % solution of quinine bihydrochloride.	
1	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
2	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 2 hours
3	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 2½ hours
4	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace at end of 3 hours

also distinct. No autolysin was present in the plasma and no malarial parasites or pigmented leucocytes were found in the blood. On examination with the aid of the haemocrit the blood was found to contain 16·3 per cent. by volume of red cells. The above observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

4th day. At 10 a.m. patient's oxalated plasma was of a deep orange colour, but not so deep as yesterday. It gave faint oxyhaemoglobin bands, 0·16 per cent. of haemoglobin being present in solution. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood.

7th day. At 10-30 a.m. patient's oxalated plasma was of a light orange colour. Oxyhaemoglobin bands were faint, the plasma containing 0·1 per cent. of haemoglobin in solution. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. On examining with the aid of the haemocrit the blood was found to contain 14·8 per cent. by volume of red cells. The following observations were made at 37° C., with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells.	0·9 % solution of sodium chloride.	1·92 % solution of quinine bihydrochloride.	
1	0·4 c.c.	2·0 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
3	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 1½ hours
4	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2 hours
5	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
6	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours
7	1·5 c.c.	6·4 c.c.	0·1 c.c.	Nil at end of 3 hours
8	1·3 c.c.	4·6 + 1 c.c. plasma	0·1 c.c.	Nil at end of 3 hours

*Condition of the spleen.*—6th day. At 11 a.m. a spleen puncture was made. Phagocytosis of red cells (by less than 0·1 per cent. of the white cells) was observed. No malarial parasites or pigment found.



*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 10.15 a.m.	225 c.c.	1.017	—	Porter coloured	Large deposit consisting of granular casts and debris and a few renal epithelial cells.	Very strong oxyhaemoglobin bands. No methaemoglobin bands.	2% (corresponding to 4.5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling 2.6%
" 11.30 "	140 c.c.	1.017	—	"	"	"	2% (corresponding to 2.8 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.6% haemoglobin.
2nd day, 10.0 a.m.	10 c.c.	1.017	—	"	"	"	2% (corresponding to 0.2 gr. of haemoglobin).
3rd day, 3.30 p.m.	10 c.c.	—	Alkaline	"	"	Both oxyhaemoglobin and methaemoglobin bands present.	Total haemoglobin passed during two days was 7.5 gr.
" 7.0 "	12 c.c.	—	"	Brownish red	"	"	1.5% (corresponding to 0.15 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling was 2.1% of haemoglobin.
4th day, 3.45 a.m.	8 c.c.	—	"	"	Deposit on centrifugalising slight, consisting of few granular casts and epithelial cells. Few red cells (about one in a field).	Very faint bands of both oxyhaemoglobin and methaemoglobin.	0.5% (corresponding to 0.05 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1% haemoglobin. Total haemoglobin passed during day was 0.2 gr. Light brown precipitate on acidifying and boiling; 3/8 column.



Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day, 7.0 a.m.	5 c.c.	—	Alkaline	Amber	Deposit as above but red cells rather more numerous.	No bands	Whitish precipitate, on acidifying and boiling, $\frac{1}{4}$ column.
" 11.0 "	10 c.c.	—	"	"	"	"	"
" 4.0 p.m.	11 c.c.	—	"	"	"	"	"
" 6.30 "	4 c.c.	—	"	"	"	"	"
" 10.0 "	10 c.c.	—	"	"	"	"	"
5th day, 6.30 a.m.	30 c.c.	1.008	"	"	Slight deposit on centrifuging consisting of granular casts, epithelial cells, red cells, and leucocytes.	"	Whitish precipitate, on acidifying and boiling, $\frac{1}{3}$ column.
" 10.0 "	10 c.c.	—	"	"	"	"	"
" 2.30 p.m.	14 c.c.	1.008	"	"	"	"	"
" 5.30 "	10 c.c.	—	"	"	"	"	"
" 7.0 "	8 c.c.	—	"	"	"	"	"
" 10.30 "	20 c.c.	1.008	"	"	"	"	"
6th day, 3.0 a.m.	12 c.c.	—	"	"	Deposit on centrifuging slight, consisting of granular casts and renal epithelial cells and a few red cells and crystals of triple phosphates.	"	Whitish precipitate on acidifying and boiling equalled $\frac{2}{3}$ column.
" 9.45 "	18 c.c.	—	"	"	"	"	"
" 7.0 p.m.	15 c.c.	—	"	"	"	"	"
" 11.25 "	16 c.c.	—	"	"	"	"	"
" 11.40 "	12 c.c.	—	"	"	"	"	"
7th day, 3.40 a.m.	18 c.c.	—	"	"	"	"	"
" 7.35 "	18 c.c.	—	"	"	"	"	"
" 9.0 "	8 c.c.	—	"	"	"	"	"
" 10.0 "	5 c.c.	—	"	"	"	"	"
" 4.0 p.m.	13 c.c.	—	"	"	"	"	"
" 6.30 "	6 c.c.	—	"	"	"	"	"
" 11.15 "	24 c.c.	—	"	"	"	"	"
8th day, 6.10 a.m.	13 c.c.	—	"	"	"	"	"
" 7.20 "	9 c.c.	—	"	"	"	"	"
" 10.0 a.m.	10 c.c.	—	"	"	"	"	"
" 1.5 p.m.	23 c.c.	—	"	"	"	"	"
" 4.50 "	20 c.c.	—	"	"	"	"	"
" 6.10 "	9 c.c.	—	"	"	"	"	"
9th day, 7.0 a.m.	8 c.c.	—	"	"	"	"	"

*Post-mortem examination.*—Body well nourished, no distinct yellowness of the skin. No oedema of the subcutaneous tissues. About 600 c.cm. of fluid in the peritoneal cavity. The spleen was considerably enlarged. The liver was enlarged and pale. The kidneys were considerably enlarged and congested; capsules slightly adherent; cortex swollen but otherwise not markedly altered; pyramids very dark, exhibiting a striated appearance; the pelvis of the kidney normal in aspect. The heart was slightly dilated; no excess of fluid in the pericardium; heart substance natural in aspect; valves of heart healthy. Pleural cavities free from fluid. Lungs not congested or oedematous. Mucous membrane of stomach and oesophagus injected. The suprarenals and pancreas presented no change. Bladder healthy in aspect; it contained about 10 c.cm. of amber coloured urine.

*Microscopical examination.*—The kidneys contained numerous brownish plugs or casts within the uriniferous and collecting tubules. These plugs were made up of coarse granules  $5\mu$  to  $3\mu$  in diameter, arranged singly or in groups, partially filling the lumen of the tubules and frequently accompanied by free epithelial cells and finely granular or flocculent deposit. Further details are given on pp. 107-119. The renal tubules were dilated. The interstitial tissue presented no change in aspect. The blood vessels contained no malarial pigment. The liver showed slight interlobular cirrhosis, but no fatty change and no malarial pigment. No malarial parasites or pigment were found in the spleen.

#### BLACKWATER FEVER. CASE 12.

Male, thirty-four years of age. Fitter. Indian.

Has been in Nyasaland for the past eleven months, having previously lived some time in Uganda. During the last six months he has suffered from fever two or three times every month. He has never previously had blackwater fever. Does not habitually take quinine, but occasionally when he has fever he takes five or ten grains. He commenced to be ill yesterday, and took five grains of quinine.

1st day. Felt no better this morning. At noon he had a rigor, and took five grains of quinine. At 4-45 p.m. he passed blackwater.

2nd day. Patient felt rather better to-day. T. 99.6° F. Spleen slightly enlarged and just palpable below costal margin. No vomiting.

3rd day. Patient appeared well to-day. The temperature was normal, and the urine amber coloured. Quinine sulphate in solution was given in three doses of one grain each.

4th day. Quinine was given as above in three doses of two grains each.

5th day. Three doses of three grains of quinine were administered to-day. The patient continued well; there was no relapse of the blackwater.

6th day. Three doses of four grains of quinine were given to-day.

7th day. From to-day the patient took five grains of quinine three times daily. The patient's recovery was uninterrupted.

*Condition of blood.*—2nd day. At 4-30 p.m. patient's oxalated plasma was of a dark orange colour. It gave distinct oxyhaemoglobin bands, 0.2 per cent. of haemoglobin being present in solution. On examining with the aid of the haemocrit, the blood was found to contain 33 per cent. by volume of red cells. On testing for haemolysin complete haemolysis occurred, but the plasma, which had been kept for twenty-four hours, was found to contain a considerable number of bacteria. No malarial parasites or pigmented leucocytes were found in the blood.

3rd day. At 10-30 a.m. patient's oxalated plasma was of an amber colour. It gave faint oxyhaemoglobin bands, when examined in a column eighteen millimetres high, 0.1 per cent. of haemoglobin being present in solution. No autolysin was present, and no malarial parasites or pigmented leucocytes were found in the blood.

*Condition of the spleen.*—2nd day. At 4-30 p.m. a spleen puncture was made. Phagocytosis of red cells (by about 3 per cent. of white cells) was observed. No malarial parasites or pigment found.

3rd day. At 10-30 a.m. a spleen puncture was made. Phagocytosis of red cells (by about 3 per cent. of white cells) was observed. No malarial parasites or pigment found.

*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 11.0 p.m.	—	1.015	Alkaline	Very dark red	Considerable deposit on centrifugalising consisting of granular casts, epithelial cells and crystals. No red cells.	Strong bands of oxyhaemoglobin and methaemoglobin.	2.2 % chocolate precipitate, on acidifying and boiling, $\frac{3}{4}$ column.
2nd day, 1.0 a.m.	—	1.008	"	"	"	"	"
" 7.0 "	200 c.c.	1.009	Acid	Claret coloured	"	"	0.34 % chocolate precipitate, on acidifying and boiling, $\frac{1}{4}$ column.
" 2.0 p.m.	—	1.012	"	Light claret coloured	"	"	0.2 %
" 4.0 "	—	1.006	"	Brownish	"	Oxyhaemoglobin but no methaemoglobin bands visible.	0.1 % chocolate precipitate, on boiling, $\frac{1}{2}$ column.
" 5.0 "	—	1.012	Alkaline	Light brown	Slight deposit consisting of granular and hyaline casts and renal epithelial cells.	"	0.8 % chocolate precipitate, on acidifying and boiling, $\frac{1}{5}$ column.
" 6.45 "	—	1.015	Acid	"	"	"	Slight precipitate on acidifying and boiling.
" 8.0 "	170 c.c.	1.014	"	Amber	Very slight deposit.	No bands.	"
3rd day	—	—	—	"	No deposit.	"	No precipitate on acidifying and boiling.

Subsequent specimens of urine were amber coloured and normal.



## BLACKWATER FEVER. CASE 13.

Male, twenty-nine years of age. Missionary. European.

Has been in Nyasaland for five years. From time to time had attacks of malaria, which were not treated with quinine. Had a slight attack of blackwater above a year ago.

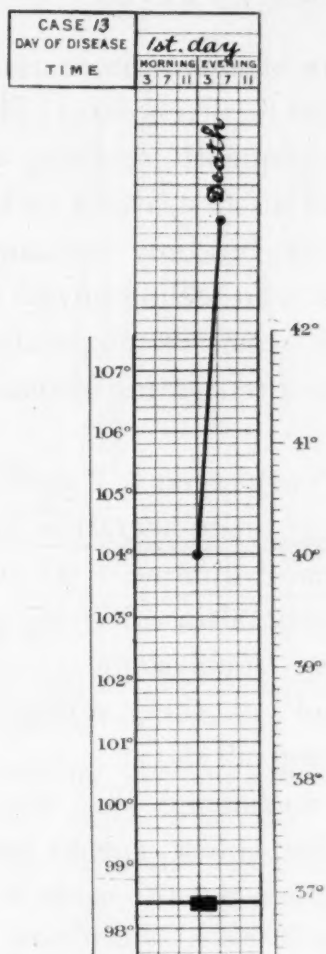


FIG 77. Blackwater Fever, Case 13. Temperature Chart.

Took four grains of quinine yesterday. Spent the day working, in a hot sun, at brick-making. Stated to have been quite well.

This morning took four grains of quinine. Remained well till mid-day. Became seriously ill at 1 p.m., when he vomited black material like coffee grounds. Temperature was then 40° C. (104° F.) He was then much distressed, and complained of pain in the abdomen, putting his hands to the hypogastric region. Passed red urine, described as 'looking like blood.' He became delirious and violent, and four men were required to restrain him. In his delirium bit the forearm of another missionary. Temperature continued to rise to

43° C. (109·4° F.). Patient's face and hands became deeply yellow. Died at 5 p.m. Not seen by myself till 11 p.m., when the urine and vomit had been thrown away. Face and hands were of a markedly brownish yellow colour. Post-mortem examination not permitted.

#### BLACKWATER FEVER. CASE 14.

Male, thirty-nine years of age. Store assistant. Indian.

Has been in Nyasaland for five years. He has had a great deal of fever, his temperature frequently reaching 102° or 103° F. About fifteen months ago he had his first attack of blackwater fever. Has not taken quinine regularly, but only occasionally when ill.

1st day of illness. Has been feeling unwell for some time, but only became seriously ill to-day, when his temperature rose to 100° F. He had a severe headache, took ten grains of quinine sulphate, and went to bed.

2nd day. Condition unchanged. T. 99·8° F. Took five grains of quinine. In the evening his temperature had risen to 101° F., and he had another five grains of quinine. At 10 p.m. he had a severe rigor. T. 105·4° F. Shortly afterwards he passed porter coloured urine. Vomiting was very troublesome.

3rd day. Patient had another severe rigor this morning. T. 106° F. Severe vomiting all day.

4th day. Condition unchanged this morning. Skin and conjunctivae of a lemon yellow colour, spleen just palpable. T. 103° F. The vomiting was still severe, but the urine was evidently clearing in the evening.

5th day. Patient much better, urine clear, vomiting less troublesome, temperature normal.

Subsequently the patient's recovery was uninterrupted.

*Condition of blood.*—4th day. At 5-30 p.m. patient's oxalated plasma was of a reddish colour; it gave in a column eighteen millimetres high distinct bands of oxyhaemoglobin and methaemoglobin, equalling 0·57 per cent. of haemoglobin in solution. No malarial parasites or pigmented leucocytes were found in the blood.

5th day. At 10-45 a.m. patient's oxalated plasma was of a deep orange colour with no red tint, containing 0·18 per cent. of haemoglobin. Methaemoglobin bands were also distinct. No autolysin

present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood. With the aid of the haemocrit the patient's blood was found to contain 33·3 per cent., by volume,

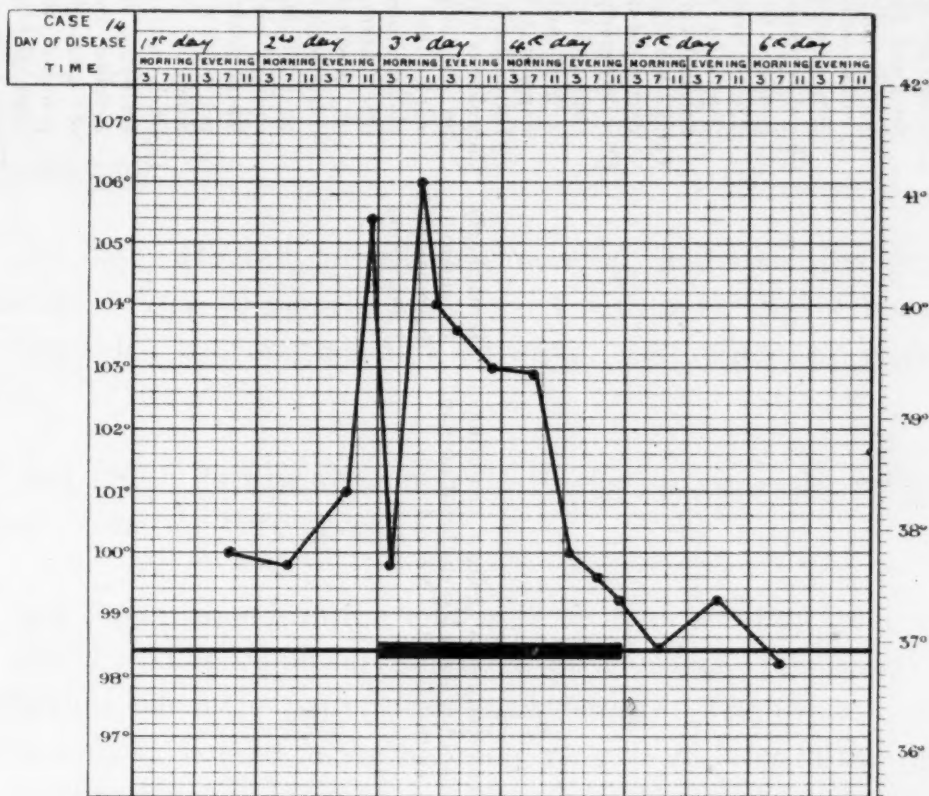


FIG. 78. Blackwater Fever, Case 14. Temperature Chart.

of red cells. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added :

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2 hours
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
5	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours

*Condition of spleen.*—4th day. At 5·30 p.m. a spleen puncture was made. Phagocytosis of red cells (by less than 1 per cent. of white cells) was observed. No malarial parasites or pigment found.

*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
3rd day, midnight until 3.0 a.m.	86 c.c.	1.026	Acid	Porter coloured	Deposit on centrifugalising slight, consisting of granular casts. No red cells.		Chocolate precipitate on acidifying and boiling less than 4.5% of haemoglobin (corresponding to 3.8 g. of haemoglobin).
3rd day, 8.30 a.m. until 5.0 p.m.	710 c.c.	1.016	"	Dark red	"		Chocolate precipitate on acidifying and boiling less than 4.25% of haemoglobin (corresponding to 3.02 g. of haemoglobin).
3rd day, 11.40 p.m.	460 c.c.	1.014	"	"	"		Chocolate precipitate on acidifying and boiling less than 3% of haemoglobin (corresponding to 13.5 g. of haemoglobin).
4th day, 9.0 a.m.	370 c.c.	1.014	"	Claret coloured	"		Total haemoglobin passed during the day was 47.5 g.
" 4.0 p.m.	310 c.c.	1.020	"	"	Deposit on centrifugalising slight, consisting of granular casts and a few epithelial casts and renal epithelial cells. No red cells.	Oxyhaemoglobin and met-haemoglobin bands strong.	Chocolate precipitate on acidifying and boiling less than 3% of haemoglobin (corresponding to 11.1 g. of haemoglobin).
" 6.0 "	400 c.c.	1.014	"	Brownish red	"	"	Chocolate coloured precipitate on acidifying and boiling was less than 3% of haemoglobin (corresponding to 9 g. of haemoglobin).
" 11.30 "	170 c.c.	1.016	"	Light brown	"	"	0.35% of wet red cells; chocolate coloured precipitate on acidifying and boiling equalled less than 1.5% of haemoglobin (corresponding to 6 g. of haemoglobin).
5th day, 3.0 a.m.	114 c.c.	1.024	"	Amber coloured	No deposit.	No bands.	Light chocolate precipitate on acidifying and boiling equalled less than 0.5% of haemoglobin (corresponding to 0.9 g. of haemoglobin).
" 8.0 "	230 c.c.	1.020	"	"	"	"	Total haemoglobin passed during the day was 27 g.
" 4.0 p.m.	—	1.016	"	"	"	"	Very slight precipitate on acidifying and boiling.
							No precipitate on acidifying and boiling.

Subsequent specimens of the urine were amber coloured and normal.



## BLACKWATER FEVER. CASE 14a.

Male, thirty-nine years of age. Store assistant. Indian.

See history of preceding attack (Case 14). Has remained in fairly good health since his last attack of blackwater fever three months ago.

1st day. He felt unwell this morning, and took ten grains of quinine. At 2-30 p.m. he was seized with a feeling of uneasiness, accompanied by vomiting and shivering. At 3 p.m. he passed red urine. T. 101.4° F.

2nd day. Patient remained ill all day, passing porter coloured urine; vomiting very severe.

3rd day. Shortly after midnight he had a rather severe rigor. T. 102° F. The vomiting continued all morning, but ceased in the afternoon, when the patient felt much better. The urine, which had been amber coloured in the morning, cleared up later in the day, and towards evening was amber in colour.

4th day. Patient much better; the temperature was normal, and the urine dark amber coloured.

Subsequently the patient's recovery was uninterrupted.

*Condition of blood.*—3rd day. At 12-30 p.m. blood was withdrawn from the finger. The blood appeared watery, and patient's oxalated plasma was of a deep orange colour with no red tint, containing 0.2 per cent. of haemoglobin in solution. Very faint bands of methaemoglobin could be detected in a column eighteen millimetres high. On examination with the aid of the haemocrit, the patient's blood was found to contain 24 per cent. by volume of red cells. A haemoglobinometer reading of 33 divisions of von Fleischl's scale was obtained (equivalent to 0.1 per cent. of wet red cells)

$$\frac{\text{Haemoglobin}}{\text{Volume}} = 1.37.$$

*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 3.0 p.m. " 8.30 "	280 c.c. 200 c.c.	—	Alkaline	Porter coloured	Deposit on centrifugalising about $\frac{1}{16}$ column of brownish white colour, consisting of granular casts, the granules being of varying degrees of coarseness and brown in colour, though not deeply tinted. Nuclei are seen in some of the casts. The longest casts measure $252 \mu \times 54 \mu$ . No red cells, stromata or leucocytes.	Oxyhaemoglobin bands but no methaemoglobin bands present.	1.8% (corresponding to 8.6 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling was less than 2.5% of haemoglobin. Total haemoglobin passed during the day was 8.6 gr.
2nd day, 3.45 a.m. " 8.30 " " 10.15 " " 2.20 p.m. " 5.30 " " 7.45 " " 12.30 a.m.	770 c.c.	—	Alkaline	Porter coloured	"	"	1.2% (corresponding to 9.3 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling less than 1.3% of haemoglobin. Total haemoglobin passed during the day was 9.3 gr.
3rd day, 1.45 a.m. " 5.30 " " 10.15 " " 12.30 p.m. " 6.0 "	150 c.c. 140 c.c. 150 c.c. 50 c.c. 120 c.c.	— — — — —	" Neutral Alkaline	" " Brownish amber coloured	" " Very little deposit on centrifugalising.	" " No bands.	0.5% (corresponding to 2.3 gr. of haemoglobin). 0.25% (corresponding to 0.1 gr. of haemoglobin). Dark brown precipitate on acidifying and boiling less than 0.4% of haemoglobin. Total haemoglobin passed during day 2.4 gr.
4th day, 6.0 a.m. " 11.45 "	610 c.c. 360 c.c.	— —	— —	Dark amber coloured Amber coloured	No deposit. "	" "	No precipitate on acidifying and boiling. "

Subsequent specimens of the urine were amber coloured and normal.

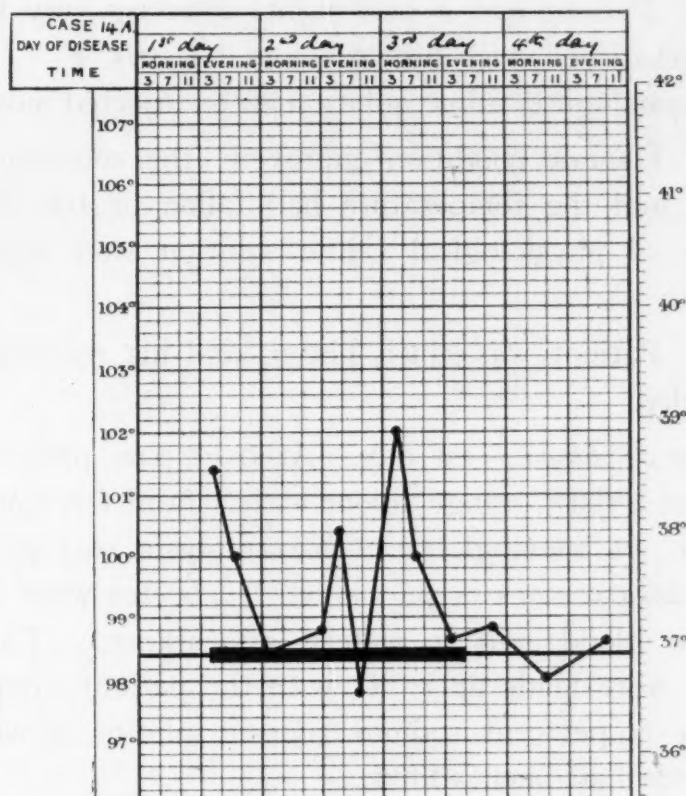


FIG. 79. Blackwater Fever, Case 14a. Temperature Chart.

#### BLACKWATER FEVER. CASE 15.

Male, twenty-five years of age. Store-keeper. European.

Has lived in Nyasaland for six months and has never been in the tropics previously. During this time he has had a good deal of fever. Stated that he had taken thirty grains of quinine a week regularly since he has been in Nyasaland, and that in spite of this he has suffered considerably from malaria. He has never used a mosquito net. Began to be ill three or four days ago. Felt feverish, had anorexia and was unable to sleep. Three days before the attack commenced he took ten grains of quinine. The next day he took five grains of quinine, and the day before the attack he took five grains of quinine.

1st day of attack. Got up about 7 a.m., and took ten grains of quinine. At 4 p.m. he noticed that his urine was porter coloured.

This was the first intimation he had that he was suffering from blackwater fever. He had no previous rigor. T. 103.6° F.

2nd day. Patient had a bad night, vomiting very troublesome, skin of a marked yellowish brown colour. T. 103° F. Twenty-three ounces of physiological saline solution were injected subcutaneously.

3rd day. General condition improved; the vomiting was not so troublesome, and the temperature had fallen to 100° F. Twenty-three ounces of physiological saline solution were again injected subcutaneously.

4th day. Patient was much better, and his recovery continued uninterruptedly.

*Condition of blood.*—1st day. At 9-30 p.m. patient's oxalated plasma was of a dark orange colour with a faint red tint, containing 0.56 per cent. of haemoglobin. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. The blood had a watery appearance. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells	0.9 % solution of sodium chloride	1.92 % solution of quinine bihydrochloride	
1	0.5 c.c.	2.4 c.c.	0.1 c.c.	Complete at end of 1 hour
2	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 2 hours
3	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 2½ hours
4	1.05 c.c.	4.6 c.c.	0.1 c.c.	Trace at end of 3 hours
5	1.3 c.c.	5.6 c.c.	0.1 c.c.	Nil at end of 3 hours

2nd day. At 1 p.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0.42 per cent. of haemoglobin. No autolysin was present in the plasma.

*Condition of the spleen.*—1st day. At 10 p.m. a spleen puncture was made. Phagocytosis of red cells (by less than 1 per cent. of white cells) was observed. No malarial parasites or pigment found.



Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 7.0 a.m.	—	—	—	Amber	—	—	—
" noon	—	—	—	Red	—	—	—
" 4.0 p.m.	—	—	—	"	—	—	—
" 6.0 "	70 c.c.	1.026	Acid	Porter coloured	Deposit on centrifugalising about $\frac{1}{10}$ column, brown coloured, consisting of granular debris and casts, also a few hyaline and epithelial casts and polygonal and columnar epithelial cells. No squamous epithelial cells and no red cells, leucocytes or bacteria.	Strong oxy-haemoglobin and methaemoglobin bands.	2% (corresponding to 1.4 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled 3.75% of haemoglobin.
" 9.10 "	89 c.c.	1.015	"	"	"	Strong oxy-haemoglobin and methaemoglobin bands.	1.3% (corresponding to 1.2 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.75 gr. of haemoglobin.
2nd day, 3.45 a.m.	360 c.c.	1.020	"	"	Deposit on centrifugalising about $\frac{1}{10}$ column, as above.	"	Total haemoglobin passed during the day was 2.6 gr. 1.3% (corresponding to 4.6 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 3% of haemoglobin.
" 7.0 a.m. until 7.10 p.m.	446 c.c.	1.016	"	"	"	"	0.7% (corresponding to 3.4 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.5% of haemoglobin.
							Total haemoglobin passed during day was 8.1 gr.

Date and time	Amount	Sp. gr.	Reaction	Colour	Appearance and amount of deposit	Spectroscopic examination	Amount of haemoglobin passed
3rd day, 5.45 a.m.	359 c.c.	1.018	Neutral	Porter coloured	Deposit on centrifugalising about $\frac{1}{10}$ column, as above.	Strong oxyhaemoglobin and methaemoglobin bands.	0.7% (corresponding to 2.5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling was 1.25% of haemoglobin.
" 7.0 "	450 c.c.	1.016	Alkaline	"	Deposit on centrifugalising $\frac{1}{8}$ column, consisting of granular debris and casts and a few epithelial casts and cells.	Oxyhaemoglobin and methaemoglobin bands present.	1.1% (corresponding to 4.5 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled less than 1.6% of haemoglobin.
" 10.30 p.m.	470 c.c.	1.015	"	Light reddish brown	Deposit on centrifugalising $\frac{1}{8}$ column as above.	Oxyhaemoglobin but no methaemoglobin bands present.	0.26% (corresponding to 1.3 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled less than 0.6% of haemoglobin. Total haemoglobin passed during the day equalled 8.3 gr.
4th day, 8.15 a.m.	900 c.c.	1.026	"	Light brown	"	"	—
" 2.20 p.m.	—	—	"	"	"	"	Whitish precipitate on acidifying and boiling.
" 5.45 "	—	—	Acid	Amber	No deposit	No bands.	Trace of precipitate on acidifying and boiling.
" 9.15 "	—	—	"	"	"	"	"

Subsequent specimens of the urine were amber coloured and normal.

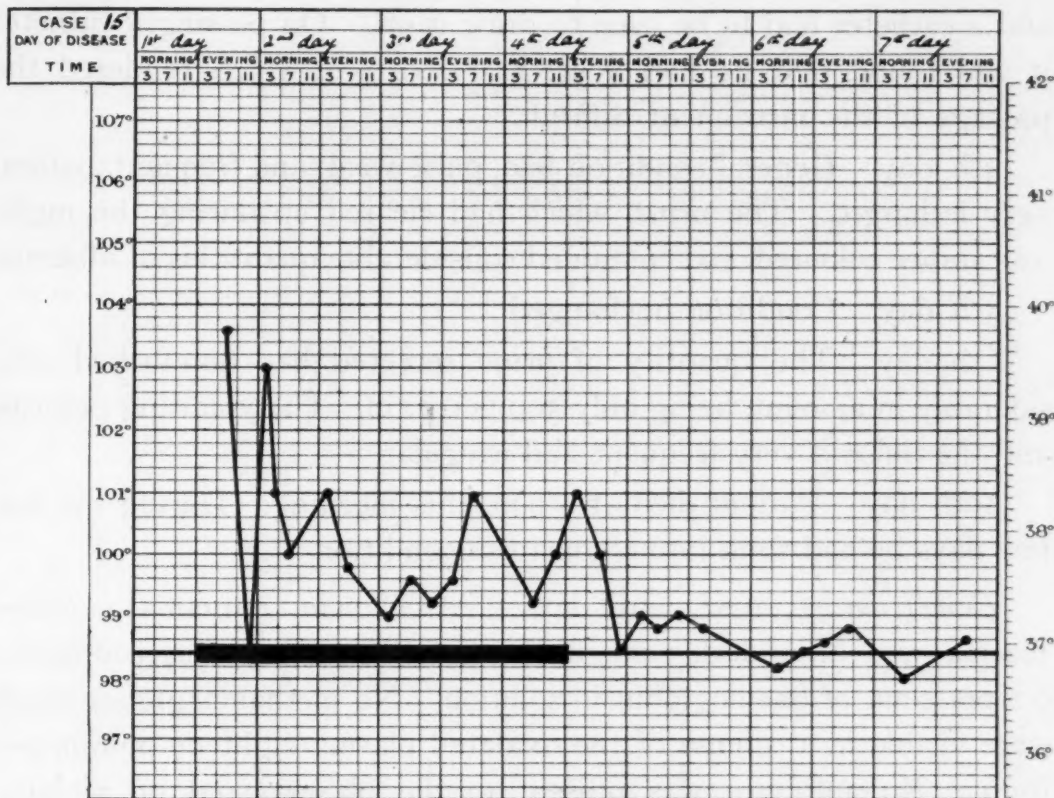


FIG. 80. Blackwater Fever, Case 15. Temperature Chart.

#### BLACKWATER FEVER. CASE 16.

Male, thirty-five years of age. Cook. Indian.

Has lived in Nyasaland for four years, and during this time has had a good deal of fever, but this has not often been so severe as to necessitate his giving up work. Has never previously had blackwater fever. Has not taken quinine regularly. He began to be ill with fever and headache four days ago. Quinine bihydrochloride was given in solution in doses of five grains three times daily. He continued to take this solution of quinine in similar doses during the next three days.

1st day. Patient passed blackwater this morning. He was obviously very ill, vomiting incessantly. The skin and conjunctivae were yellowish. T. 101.6° F. He had considerable diarrhoea, and during the day he passed six black tarry stools and stated he had been passing similar stools for the past three or four days.

2nd day. His condition was unchanged. The spleen could just be felt below the costal margin.

3rd day. The urine was still red coloured, and the vomiting was very troublesome. After to-day patient was unable to pass his urine, and a catheter had to be used to draw it off. On passing a catheter it was found that he had a urethral stricture, which rendered the passage of the instrument difficult.

4th day. General condition bad, pulse weak and frequent, patient very lethargic. The urine, which had cleared up during the night, was amber coloured, and contained considerable quantities of albumin.

5th day. Condition unchanged.

6th day. The quantity of urine secreted has diminished considerably in amount, being only 500-600 c.cm. The vomiting persists, and the pulse is very frequent and irregular.

10th day. Patient died at 1 a.m. this morning. During the last few days he had sunk into a comatose condition.

*Condition of blood.*—3rd day. At 10 a.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0.2 per cent. of haemoglobin in solution. No methaemoglobin bands were visible in a column of the oxalated plasma eighteen millimetres high. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0.9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2.5 % emulsion of red blood cells.	0.9 % solution of sodium chloride.	1.92 % solution of quinine bihydrochloride.	
1	0.65 c.c.	3.0 c.c.	0.1 c.c.	Complete at end of 2 hours.
2	0.8 c.c.	3.6 c.c.	0.1 c.c.	Complete at end of 2½ hours.
3	1.05 c.c.	4.6 c.c.	0.1 c.c.	Partial at end of 3 hours.
4	1.3 c.c.	5.6 c.c.	0.1 c.c.	Trace at end of 3 hours.

*Condition of spleen.*—3rd day. At 11 a.m. a spleen puncture was made. Phagocytosis of red cells (by about 1 per cent. of the white cells) was observed. No malarial parasites or pigment found.



*Condition of urine.*

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day	560 c.c.	—	—	Porter coloured			
2nd day (evening)	840 c.c.	—	—	Reddish brown	Slight whitish deposit on centrifugalising, consisting of granular casts and epithelial cells. No red cells.	Oxyhaemoglobin and methaemoglobin bands present.	Chocolate coloured precipitate on acidifying and boiling equalled 0.5 % of haemoglobin. Total amount of haemoglobin passed during the day was 4.2 gr.
3rd day, 8.0 a.m.) " 9.0 "	336 c.c.	1.016	Alkaline	Red	"	"	Chocolate coloured precipitate on acidifying and boiling equalled 0.7 % haemoglobin. Total amount of haemoglobin passed during the day was 2.5 gr.
4th day	336 c.c.	1.015	Acid	Amber	Very slight deposit, as above.	No bands	White precipitate on acidifying and boiling equalled 1.6 column.
5th day	544 c.c.	1.015	"	"	"	"	"
6th day	500 c.c.	—	—	"	"	"	"
7th day	1400 c.c.	—	—	"	"	"	"
8th day	840 c.c.	—	—	"	"	"	"
9th day	420 c.c.	—	—	"	"	"	"

*Post-mortem examination.*—Body fairly well nourished. Skin sallow. No signs of decomposition. Rigor mortis well marked. No excess of fluid in the pericardial cavity; heart muscle healthy; valves of heart normal; no atheroma of aorta. Pleura free from adhesions, no effusion in pleural cavities. Lungs markedly congested and oedematous, no tubercle present. No fluid in the peritoneal cavity. Liver healthy in aspect. Spleen slightly enlarged. The kidneys

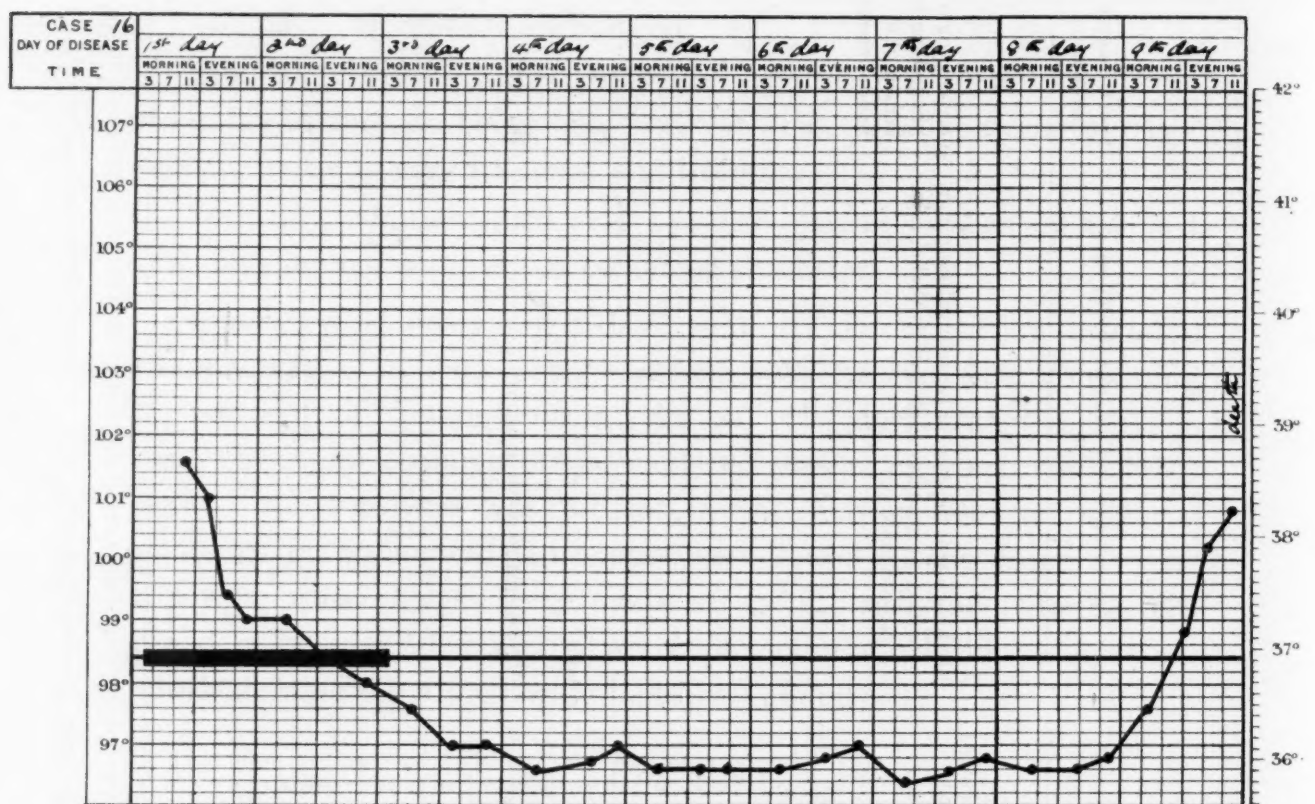


FIG. 81. Blackwater Fever, Case 16. Temperature Chart.

were slightly enlarged; capsules stripped readily; cortex not markedly altered in naked eye aspect; pyramids dark, presenting a striated appearance on section, the collecting tubules being easily seen with the naked eye; pelvis of kidney normal. Suprarenals and pancreas normal. Stomach and intestines normal. Bladder, which contained a little amber coloured urine, was normal in appearance. The brain and its membranes presented no macroscopic change.

*Microscopic examination.* The kidneys exhibited in many of the uriniferous and collecting tubules plugs of brownish material made up of granules,  $5\mu$  to  $2\mu$  or less in diameter, and granular masses,

together with epithelial cells and flocculent debris; further details are given on pp. 119-123. The lumen of the uriniferous tubules was wide, but did not exhibit the marked distension seen in the kidneys of Case 7a. The interstitial tissue was free from haemorrhage, and showed no cell infiltration or increase of connective tissue. No cirrhosis or fatty degeneration of the liver or malarial pigment in its vessels. No malarial parasites or pigment was found in the spleen.

#### BLACKWATER FEVER. CASE 17.

Male, thirty-two years of age. Planter. European.

Has lived in Nyasaland for ten years, during which time he has had one or two attacks of fever every wet season. In the course of the last eight years he has had four attacks of blackwater fever. He only occasionally takes quinine. He suffered from an attack of fever four days ago. T.  $105^{\circ}$  F. Took twenty grains of quinine. He remained ill for the next three days, and had fifteen grains of quinine each day.

1st day. Was still ill, and took ten grains of quinine in the morning. During the evening he noticed that he was passing blackwater. He was brought into hospital the same evening. His condition was one of considerable exhaustion, with troublesome vomiting. Spleen slightly enlarged, and just palpable. T.  $101^{\circ}$  F.

2nd day. His condition to-day was unchanged, and the vomiting continued all day. T.  $105^{\circ}$  F. This rise in temperature was accompanied by a very severe rigor. The urine was porter coloured.

3rd day. The patient was rather better this morning and the temperature almost normal. The urine was still highly coloured.

4th day. The urine became decidedly lighter towards evening.

5th day. Patient feels quite well. Urine almost clear.

6th day. The urine was amber coloured. Patient's subsequent recovery was uninterrupted.

*Condition of blood.*—1st day. At 11-30 p.m. patient's oxalated plasma was of a deep orange colour, with a distinct red tint, containing 0.65 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood.



2nd day. At 9 a.m. patient's oxalated plasma was of a reddish orange colour, containing 0.67 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood. At 3-15 p.m. patient's oxalated plasma was of a reddish orange colour, containing 0.95 per cent. of haemoglobin in solution.

3rd day. At 10-30 a.m. patient's oxalated plasma was of a reddish orange colour, containing 0.48 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood.

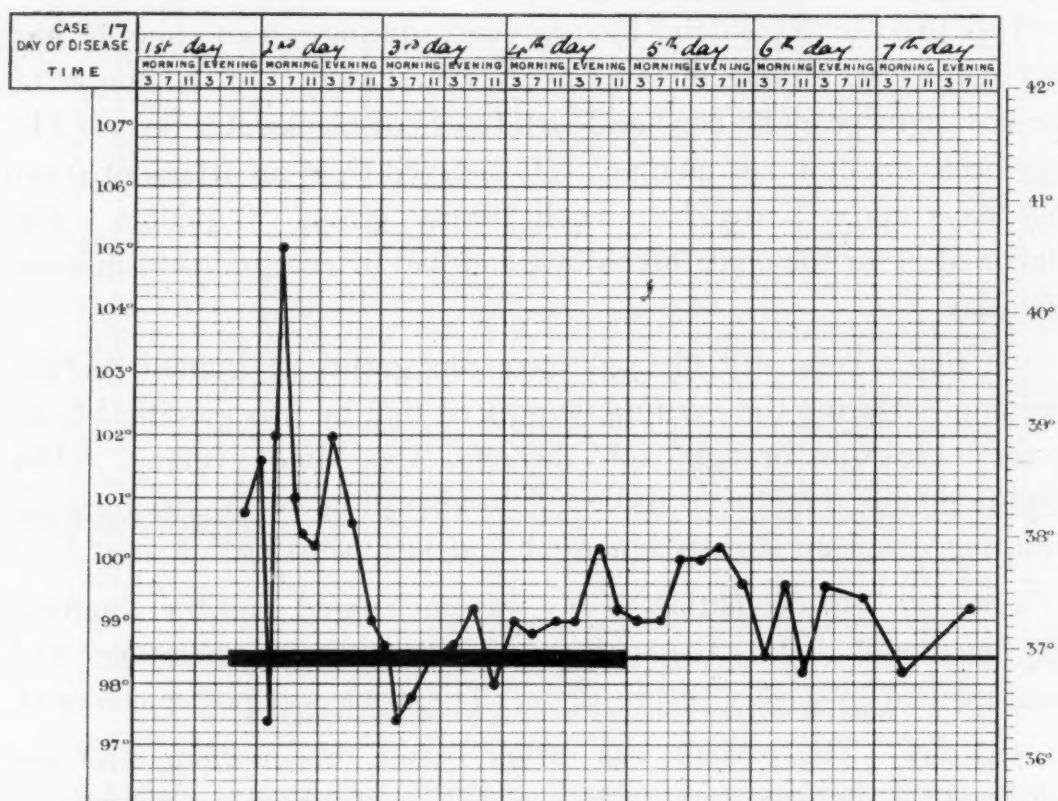


FIG. 82. Blackwater Fever, Case 17. Temperature Chart.

4th day. At 11-45 a.m. patient's oxalated plasma was of a deep orange colour with no red tint, containing 0.18 per cent. of haemoglobin in solution. On examining the blood with the aid of the haemocrit, it was found to contain 22.5 per cent. by volume of red cells. A haemoglobinometer reading of 21.5 divisions of von Fleischl's scale was obtained (equivalent to 0.07 per cent. of wet red cells).



Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 11.0 p.m.	285 c.c.	1.020	Acid	Claret coloured	—	Oxyhaemoglobin and methaemoglobin bands present.	1.45% (corresponding to 3.6 gr. of haemoglobin). Total haemoglobin passed during the day was 3.6 gr.
2nd day, 12.30 a.m.	28 c.c.	1.015	"	Dark red coloured	Slight deposit on centrifugalising consisting of granular casts and epithelial cells and red cells.	Oxyhaemoglobin bands present but no methaemoglobin bands.	3% (corresponding to 0.8 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling was 3.5% of haemoglobin.
" 4.0 "	85.5 c.c.	—	—	"	"	"	1.9% (corresponding to 1.5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 3% of haemoglobin.
" 4.30 "	28 c.c.	—	Alkaline	"	"	"	1.5% (corresponding to 0.45 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling 2.5% of haemoglobin.
" 7.0 "	171 c.c.	1.014	"	"	"	Oxyhaemoglobin and methaemoglobin bands present.	1.3% (corresponding to 2.4 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled 1.3% of haemoglobin.
" 9.30 "	142.5 c.c.	—	"	Porter coloured	Deposit on centrifugalising about $\frac{1}{3}$ th column consisting of granular casts, epithelial cells and casts. No red cells.	"	1.4% (corresponding to 1.5 gr. of haemoglobin).
" 12.15 p.m.	142 c.c.	1.014	"	"	"	"	1.4% (corresponding to 1.5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling 2.5% of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
2nd day, 1.45 p.m.	171 c.c.	1.014	Alkaline	Dark red	Deposit on centrifugalising about $\frac{3}{8}$ th column consisting of granular casts, epithelial cells and casts. No red cells.	Oxyhaemoglobin and met-haemoglobin bands present.	1.5% (corresponding to 2.5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling 2.5% of haemoglobin.
" 3.10 "	228 c.c.	1.012	"	"	"	"	2.0% (corresponding to 4.5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.5% of haemoglobin.
" 5.15 "	171 c.c.	1.012	"	"	"	"	1.6% (corresponding to 2.7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.5% of haemoglobin.
3rd day, 4.15 a.m.	85.5 c.c.	—	"	Dark reddish brown	"	"	Total haemoglobin passed during the day was 17.9 g. of haemoglobin. Chocolate precipitate on acidifying and boiling equalled 2.3% of haemoglobin.
" 6.10 "	70 c.c.	—	"	Reddish brown	"	"	"
" 9.5 "	114 c.c.	—	"	Porter coloured	Deposit on centrifugalising $\frac{1}{2}$ th column, consisting of granular casts and epithelial casts, and renal epithelial cells and few red cells.	"	0.5% (corresponding to 0.6 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.3% of haemoglobin.
" 11.20 "	57 c.c.	1.014	Acid	"	"	"	0.4% (corresponding to 0.2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2.0% of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
3rd day, 12.30 p.m.	28.5 c.c.	1.012	Acid	Porter coloured	Deposit on centrifugalising $\frac{1}{2}$ column, consisting of granular casts and epithelial cells, and renal epithelial cells and few red cells.	Oxyhaemoglobin and met-haemoglobin bands present.	0.3% (corresponding to 0.1 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2% of haemoglobin.
" 1.10 "	28.5 c.c.	1.012	"	"	"	"	0.25% (corresponding to 0.7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1.5% of haemoglobin.
" 3.0 "	14.2 c.c.	1.012	"	"	"	"	0.36% (corresponding to 0.7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2% of haemoglobin.
" 4.0 "	11.4 c.c.	1.011	"	"	"	"	0.25% (corresponding to 0.3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1.7% of haemoglobin.
" 5.0 "	25.6 c.c.	1.010	"	"	Deposit on centrifugalising slight, consisting of renal epithelial cells in large numbers and a few granular and epithelial casts.	"	0.2% (corresponding to 0.5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1.7% of haemoglobin.
" 6.15 "	28.5 c.c.	1.005	"	"	"	"	0.15% (corresponding to 0.4 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1.2% of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
3rd day, 7.0 p.m.	228 c.c.	1.008	Acid	Porter coloured	Deposit on centrifugalising slight, consisting of renal epithelial cells in large numbers and a few granular and epithelial casts.	Oxyhaemoglobin and met-haemoglobin bands present.	0.15% (corresponding to 0.3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1.2% of haemoglobin.
" 8.30 "	228 c.c.	1.014	"	Reddish brown	"	Oxyhaemoglobin bands present, met-haemoglobin bands absent.	0.1% (corresponding to 0.2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0.7% of haemoglobin.
" 9.30 "	114 c.c.	1.014	"	"	"	"	0.1% (corresponding to 0.1 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1% of haemoglobin.
" 11.45 "	200 c.c.	1.010	"	Brown	"	"	0.1% (corresponding to 0.2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0.5% of haemoglobin.
4th day, 3.0 a.m.	342 c.c.	1.010	"	"	"	"	Total haemoglobin passed during day was 4.2 g. 0.1% (corresponding to 0.3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0.5% of haemoglobin.
" 6.15 "	314 c.c.	1.009	"	"	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts and red cells.	"	0.1% (corresponding to 0.3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0.5% of haemoglobin.



Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day, 9.40 a.m.	400 c.c.	1.009	Acid	Brown	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts and red cells.	Oxyhaemoglobin bands present, met-haemoglobin bands absent.	0.1 % (corresponding to 0.4 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0.5 % of haemoglobin.
" 2.0 p.m.	456 c.c.	1.010	"	Light brown	"	"	0.06 % (corresponding to 0.3 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.5 % of haemoglobin.
" 4.30 "	285 c.c.	1.009	"	"	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts.	"	0.07 % (corresponding to 0.2 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.5 % of haemoglobin.
" 7.30 "	228 c.c.	1.012	"	Dark amber coloured	"	"	0.05 % (corresponding to 0.1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4 % of haemoglobin.
" 10.0 "	170 c.c.	1.012	"	"	"	"	0.06 % (corresponding to 0.1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4 % of haemoglobin.
" 11.45 "	340 c.c.	1.012	"	"	"	"	0.05 % (corresponding to 0.17 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.3 % of haemoglobin. Total haemoglobin passed during the day was 1.8 g.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
5th day, 2.0 a.m.	170 c.c.	1.010	Alkaline	Dark amber coloured	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts.	Oxyhaemoglobin bands present, met-haemoglobin bands absent.	0.06% (corresponding to 0.1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4% of haemoglobin.
" 6.30 "	430 c.c.	1.010	"	"	Deposit on centrifugalising slight, consisting of numerous renal epithelial cells with few granular casts.	"	0.05% (corresponding to 0.2 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4% of haemoglobin.
" 10.45 "	228 c.c.	1.009	"	"	"	"	0.06% (corresponding to 0.1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4% of haemoglobin.
" 12.20 p.m.	256 c.c.	1.010	"	Brownish	"	"	0.1% (corresponding to 0.25 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0.4% of haemoglobin.
" 1.30 "	85 c.c.	1.010	"	Deep amber coloured	"	"	Light brown precipitate on acidifying and boiling equalled 0.3% of haemoglobin.
" 2.30 "	228 c.c.	1.010	"	"	"	"	"
" 4.30 "	—	—	—	Amber coloured	No deposit	No bands.	Slight white precipitate on acidifying and boiling.
" 7.30 "	—	—	—	"	"	"	"
" 11.0 "	—	—	Acid	"	"	"	No precipitate on acidifying and boiling. Total haemoglobin passed during the day was 0.8 g.

Subsequent specimens of the urine were amber coloured and normal.







TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	
4	Second attack	1st day							Dark red.				
		2nd "							"				
		3rd "							Red to amber coloured.				
		4th "							Amber coloured.	Absent			
		5th "				None found			"	"		No suspended matter.	
		6th "			0.13 %	"			"	"		"	
		7th "	Dark orange	No isolysin					Dark amber coloured.	"		"	
		8th "							Amber coloured.	"		"	
		14th "							"	"		"	
									Burgundy red.				
5	Second attack	1st "							Yellow.				
		2nd "							"				
		3rd "							Port wine red.				
		4th "							Claret red.				
		5th "							Port wine red to reddish amber.	Present		Brown precipitate : granular casts.	
		6th "	Orange	No autolysin	0.13 %	"	About 0.045 % (Compare Table 24).		Brownish amber.	Absent		No suspended matter.	

TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				Change in quantity.	
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemo-globin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.		
6	Third attack	1st day						Porter coloured.						
		2nd "						"						
		3rd "	Dark orange	No isolysin	0.16 %	None found			Faint red to amber coloured.		Present	Granular casts and renal epithelial cells.		
		4th "							Amber coloured.		No suspended matter.			
6a	Fourth attack	1st "						Porter coloured.						
		2nd "						Brown.		No oxyhaemo-globin bands.	"	Granular casts and renal epithelial cells.		
		3rd "	Pale orange	No autolysin	0.16 %	"		"	"	"	"	"		
		4th "						"	"	"	"	"		
		5th "						Amber coloured.	"	"	"	"		
7	Second attack	1st "						"						
		2nd "						"						
		3rd "						"						
		4th "	Dark orange	"	Less than 0.13 %		About 0.045 % (Compare Table 24)	1.18	Porter coloured.	"	Absent	Abundant chocolate coloured precipitate : brown granules and granular casts.		
		5th "							Dark amber coloured.	"	"	"		
		6th "							Amber coloured.		"	Chocolate coloured precipitate : brown granules and granular casts.		
		7th "	Light orange		0.06 %	"			"		"	"		

Table 52.—(Continued.) Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.				CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemo- globin.	Parasites (about 5000 red cells examined).	Smallest con- centration of quinine bi- hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemo- globin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.		
7a	Third attack	1st day	Dark orange with reddish tint	No autolysin	0.74 %	None found	0.053 %		Porter coloured.	0.4 % to 1.2 %	Present	Brownish black deposit : red cells, granular casts.	437 c.c.	
		2nd "							Port wine to yellow.	1.2 % to 0.0 %	"	"	18 c.c.	
		3rd "							"		"	Slight light yellow deposit : granular casts, renal epi- thelium.	21 c.c.	
		4th "							"		"	"	11 c.c.	
		5th "							"		"	"	17 c.c.	
		6th "							"		"	"	33 c.c.	
		7th "							"		"	"	48 c.c.	
		8th "							"		"	"	41 c.c.	
		9th "							"		"	"	23 c.c.	
		10th "							"		"	"	43 c.c.	
8	First attack	1st "							"					
		2nd "							"					
		3rd "							"					
		4th "							Porter coloured.					
		5th "	Deep orange	"	0.13 %	"	0.045 %		Deep red to light amber.	0.2 %	"	Granular casts, renal epi- thelial casts.		
		6th "	"		0.07 %	"	0.045 %		Amber coloured.		Absent	No suspended matter.		
		7th "							Red.					
		8th "							Amber coloured.					

TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.				CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.		
9	First attack	1st day												
		2nd "												
		3rd "												
		4th "												
		5th "												
		6th "												
		7th "												
		8th "	Light yellow	Complete haemolysis	Less than 0.06 %	None found	0.045 %						Pus cells, squamous epithelial cells, granular casts and masses.	
		9th "												



TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	
10	First attack	1st day											
		2nd "	Dark orange with reddish tint	No autolysin	0.85 %	None found	0.045 %	1.01	Porter coloured.	0.6 %	Absent	Much chocolate deposit : granular casts and masses.	
		3rd "	Slightly lighter orange		0.09 %	"		1.18	Porter coloured to amber coloured.	1.1 %	Present	Much chocolate deposit : granular casts and masses and epithelial cells.	
		4th "							"	Trace	—	Much chocolate deposit : granular casts and masses.	
		5th "	Deep orange		0.30 %	"	0.053 %		"	0.8 %	Present	"	
		6th "	"		0.20 %	"			"	1.6 %	Absent	Chocolate deposit : granular casts and masses and epithelial cells.	
		7th "				"			Light red to amber coloured.	0.4 %	"	Chocolate deposit : granular casts and masses.	
		8th "				"			Porter coloured to amber coloured.	0.8 %	"	"	
		9th "				"			"	0.8 %	"	"	
		10th "				"			Light red to amber coloured.	1.0 %	"	"	
		11th "				"			"	2.1 %	"	"	
		12th "				"			Amber coloured.	Absent	"	"	
		13th "				"			Red to amber coloured.	0.8 %	"	Brown deposit : granular casts and masses.	
		14th "				"			Brownish red to amber coloured.	0.6 %	"	"	
		15th "				"			Brownish amber coloured.	Trace	"	"	
		16th "				"			"	Absent	"	"	
						"			Amber coloured.	"	"	"	

TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	
11	First attack	1st day							Porter coloured.		Absent	Much deposit : granular casts, epithelial cells.	370 c.c.
		2nd "							"		"	"	362 c.c.
		3rd "	Dark orange	No autolysin	0.25 %	None found	0.045 %		Dark red to light red coloured.	1.5 % to 0.5 %	"	"	
		4th "	Slightly lighter	"	0.16 %	"		Brown-red to amber coloured.	Trace	Present	Slight deposit : granular casts, epithelial cells.	48 c.c.	
		5th "						Amber coloured.		"	"	"	92 c.c.
		6th "						"		"	"	"	73 c.c.
		7th "	Light orange	"	0.10 %	"	0.045 %		"		"	Very slight deposit : granular casts, epithelial cells.	93 c.c.
		8th "				"			"		"	"	85 c.c.
		9th "							"		"	"	8 c.c.
12	First attack	1st day							Very dark red.	2.2 %	Absent	Considerable deposit : granular casts, renal epithelial cells.	
		2nd "	Dark orange	Complete haemolysis	0.21 %	"		Claret coloured to amber coloured.	0.3 %	"	"	"	
		3rd "	Orange	No autolysin	0.14 %	"		Amber coloured.		"	No deposit.		
		4th "						"		"	"	"	
13	Second attack	1st "							Like blood.				

TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.			Change in quantity.	
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.		Suspended matter.
14	Second attack	1st day							Amber coloured.				
		2nd "							"				
		3rd "							Porter coloured.		Absent		
		4th "	Light red		0.57 %	None found			Claret coloured.	3.0 % to 0.5 %	"	Slight deposit : granular casts, epithelial cells.	
		5th "	Deep orange	No autolysin	0.18 %	"	0.045 %		Amber coloured.		"	Slight deposit : granular casts, renal epithelial cells.	
		6th "							"				
14a	Third attack	1st "							Porter coloured.	1.8 %		Brownish white deposit : granular casts and masses.	
		2nd "			0.20 %	"			"	1.2 %	"	"	
		3rd "	Dark orange					1.37	Brownish amber.	0.5 %	"	"	
		4th "							Amber coloured.		"		
15	First attack	1st "	"	"	0.56 %	"	0.045 %		Amber to porter coloured.	0.2 %	"	Brownish deposit : granular casts and epithelial cells.	
		2nd "	"	"	0.42 %	"			Porter coloured.	1.3 % to 0.7 %	"	Brownish deposit : granular casts, epithelial cells.	
		3rd "							"	1.1 % to 0.3 %	"	"	
		4th "							Light brown to amber coloured.		"	"	
		5th "							Amber coloured.		"	"	

TABLE 52.—(Continued). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				Change in quantity.
			Colour.	Haemolysin.	Amount of dissolved haemo-globin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemo-globin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	
16	First attack	1st day							Porter coloured.		Absent		560 c.c.
		2nd "							Red-brown.	0.5 %	"	Slight whitish deposit: granular casts, renal epithelial cells.	840 c.c.
		3rd "	Dark orange	No autolysin	0.20 %	None found	0.045 %		Reddish to amber coloured.	0.7 %	"	"	336 c.c.
		4th "							Amber coloured.		"		336 c.c.
		5th "							"		"		544 c.c.
		6th "							"		"		500 c.c.
		7th "							"		"		1400 c.c.
		8th "							"		"		840 c.c.
		9th "							"		"		420 c.c.
17	Fifth attack	1st "	Dark orange		0.65 % 0.67 %	"		Claret red.	1.5 %	Present	Chocolate coloured deposit: epithelial cells, granular casts.		
		2nd "	Dark orange with reddish tint		0.95 %	"		Porter coloured.	3.1 % to 1.3 %	"	"	"	
		3rd "	"		0.48 %	"		"	1.3 % to 0.3 %	"	"	"	
		4th "	"		0.18 %	"		Brown.	0.3 % or less	"	"	"	
		5th "	Dark orange			"		Amber coloured.					



## PREFACE

The members of this expedition\* desire to offer on behalf of this Tropical School, and indeed of all interested in trypanosomiasis, their grateful thanks to the British South Africa Company, the administrators of Rhodesia, for the enlightened way in which they undertook this survey of that territory, and to Sir Alfred Sharpe, K.C.M.G., the Governor of Nyasaland, for his ready and sympathetic assistance on questions relating to the country more immediately under his control.

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A. K.

R. E. M.

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\* *The eighteenth expedition of the School, to the Zambesi, 1907-1909. Consisting of A. Kinghorn and R. E. Montgomery.*



# ON THE FLAGELLATES OCCURRING IN THE INTESTINE OF *GLOSSINA PAL- PALIS* AND IN THE INTESTINE AND PROBOSCIS OF *GLOSSINA MORSITANS*

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	CONTENTS	PAGE
I.	Introduction .....	260
II.	Where work was done .....	261
III.	Methods .....	261
IV.	Incidence of intestinal infection.....	262
	(A) in <i>Gl. palpalis</i> .....	262
	(B) in <i>Gl. morsitans</i> .....	263
V.	Incidence of infection of proboscis ( <i>Gl. morsitans</i> ).....	264
VI.	Morphology of forms found in <i>Gl. palpalis</i> .....	265
VII.	Morphology of forms found in <i>Gl. morsitans</i> .....	268
	(A) in gut .....	268
	(B) in proboscis .....	270
VIII.	Feeding experiments .....	271
IX.	Origin of the parasites .....	273
X.	Literature .....	276

## I. INTRODUCTION

The following notes form a record of some observations which were made on the parasites occurring in the intestine of *Glossina palpalis* and in the intestine and proboscis of *Glossina morsitans*. They are admittedly scanty and incomplete, as we were able to devote only a very short time to the work, and much of this time was disadvantageously employed through the non-receipt of literature bearing on what had already been done on these protozoa.

In the earliest efforts made to trace a developmental cycle of the pathogenic trypanosomes in the tsetse flies, e.g., in the work of Koch,<sup>1</sup> and Gray and Tulloch,<sup>2</sup> the not unnatural mistake was made of considering all the parasites found in the flies to be derived from the pathogenic forms. As a result of further work done in conjunction with Minchin,<sup>3</sup> Gray and Tulloch admitted that their earlier conclusions were erroneous, and about the same time Novy<sup>4</sup> pointed out the possibility of the same thing. Stuhlmann<sup>5</sup> took the precaution of working with laboratory-bred flies, but in a recent paper, in which the whole subject is reviewed, Patton and Strickland<sup>6</sup> consider that sources of error exist in his experiments, and they also take exception to some of Minchin's<sup>7</sup> results and to those of Keysselitz and Mayer,<sup>8</sup> Roubaud,<sup>9</sup> and others.

In the light of our present knowledge of the wide-spread infection of many arthropods by various protozoal parasites there are some grounds for the criticism of much of the previous work. It is therefore apparent that in approaching a subject beset by so many inherent difficulties the greatest care must be exercised in the conduct of the work and that it must be controlled in the most complete manner. The use of 'wild' tsetse flies in work on development cannot be justified on any ground; for, apart from the presence of benign parasites which confuse the results, there exists a possibility that they may be naturally infected with a different species of trypanosome from the one which is being employed in the experiments. Even in the case of laboratory-fed flies, the criticism has been made that sufficient care has not been observed to exclude the occurrence of a hereditary transmission of the non-pathogenic parasites. Minchin and Stuhlmann dispute the possibility of this, while Novy and McNeal and Patton and Strickland think that it may occur.



## II. CONDITIONS UNDER WHICH WORK WAS DONE

Our observations, in the case of *Glossina palpalis* (var. *wellmani*), were carried out on Matondwi Island at the southern extremity of Lake Tanganyika. This island lies between two and three miles from the mainland, and measures about one mile in length by half a mile in width. It slopes with more or less abruptness from the water to a height of about one hundred feet, and is composed of rough broken stones on which grows a thin scrubby type of bush. The shores are similar to those of the lake in that they present at some places dense growths of reeds, at others scattered mimosa trees, and at others bare rocky stretches devoid of vegetation. *Glossina palpalis* was very abundant along the shore, and large numbers were caught daily without any difficulty.

For more than the past twenty years the island has been uninhabited, and during this period has practically never been visited even temporarily by the natives of the mainland. The fauna is composed of some crocodiles, large numbers of birds, principally water-species (e.g., darters, egrets, herons, &c.), a few snakes, and a species of mouse; no other mammalia exist, save perhaps an occasional visiting hippopotamus.

During the month of July, 1908 (middle of dry season) we spent four weeks on Matondwi, and in November, 1908 (just after the rains had commenced), returned for a three days' visit.

The work with *Glossina morsitans* was done at a camp near Kambole, about 50 miles west of Abercorn, during the month of October, 1908 (end of dry season). In this immediate neighbourhood, game is very scarce at that time of the year. Only an occasional duiker was seen while we were at this place.

## III. METHODS

We found the best method of extracting the intestine was as follows.

The fly was laid, dorsal surface downwards, on a clean slide, so that the last segment nearly touched a large drop of salt-citrate solution (NaCl and Sodium Citrate  $\bar{a}$   $\bar{a}$  0.5 per cent.) in the centre of the glass. A mounted needle was then pressed against the posterior surface of

the thorax, and traction was made on the everted hypopygium in the male, or on the last abdominal segment of the female, with a second needle. The last segment was thus separated from the rest of the abdomen and with it the attached gut was pulled out into the drop of fluid. With a little care the whole intestine, including the oesophageal portion, could be obtained intact and quite free from the other abdominal contents. The malpighian tubules were usually the only other organs which came away with the gut; sometimes spermathecae, ovaries, and uterus. This procedure, as will be seen, varies slightly from that given by Minchin,<sup>7</sup> in which the terminal segment of the fly was snipped off and the whole of the abdominal viscera expressed.

The whole intestine was mounted in the salt-citrate solution and examined under the low and high powers. Fresh preparations and smears were afterwards made from the various portions of the intestine and other abdominal contents. The smears were fixed in absolute alcohol and Fleming's solution and stained by Giemsa's and Leishman's methods.

The proboscis was examined in salt-citrate solution, and smears were made and stained as in the case of the intestinal contents.

#### IV. INCIDENCE OF INTESTINAL CONTAMINATION

##### A. *Glossina palpalis*.

During July, 1,409 *Gl. palpalis* were caught, 1,282 males and 127 females. Of these, 185 were dissected and examined, and 78 were found to harbour intestinal protozoa, a percentage of 42.1. This is by far the highest percentage of tsetse flies which has yet been recorded as containing these trypanosomes. In the Sesse Islands 11 per cent. were infected, in the French Congo about 10 per cent., in the Congo Free State infection is apparently unknown, according to Dutton, Todd and Hanington.\*<sup>10</sup>

The comparative ratio of infection in the two sexes was approximately the same.

				Positive				Negative				Percentage
Males	...	...	...	73	...	...	...	96	...	...	...	43.1
Females	...	...	...	5	...	...	...	11	...	...	...	37.5

\* This is rather questionable, however, judging from the extremely widespread occurrence of the condition.

It was observed also that the ratios of infection amongst batches of flies caught in various sections of the island were markedly different, e.g. :—

Section				Positive		Negative		Percentage
A	...	...	...	47	...	40	...	54
B	...	...	...	7	...	22	...	24.1
C	...	...	...	24	...	45	...	34.9

No apparent reason existed for these discrepancies. Section A, the most heavily infected, was directly opposite the camp, and our natives were constantly going and coming from the water along this stretch, so that it was avoided by all the birds. No crocodiles were ever seen along this bit of the shore.

About a third (33.1 per cent.) of the flies which were examined showed evidence of having fed on blood (61 out of 185). In 25, oval, nucleated cells were seen; in 35, the pigment and detritus left from digested blood; and in only one, fresh mammalian blood was found, evidently from one of the boys engaged in catching the flies.

In November, 401 flies were caught, 396 males and 5 females. One hundred of these were examined, 99 males and 1 female, and 41 were found to be infected.

				Positive		Negative		Percentage
Males	...	...	...	41	...	58	...	41.4
Females	...	...	...	0	...	1	...	0

At this time special attention was devoted to the proboscis, but in no case were parasites found in this part of the flies.

#### B. *Glossina morsitans*.

Of 365 flies, 313 were males and 52 females, a proportion of 6 : 1. One hundred and thirteen were examined, and of these 32.8 per cent. showed signs of having fed on blood (in 3 mammalian blood corpuscles were observed, and in 34 blood pigment), practically the same percentage as noted in the case of *Gl. palpalis*. All traces of blood disappear from the intestine of *Gl. morsitans* in about 96 hours, and it is altogether probable that, if the flies feed on blood alone, they are capable of remaining alive without it for some considerable period. The condition of the abdominal organs of many of the flies which were examined was such as to render it perfectly clear that much longer than four days had elapsed since last they had fed on blood.



The possibility of tsetse flies feeding on anything except blood has recently been mooted again.<sup>11</sup> The following observation, though negative, may be worth noting.

On one occasion at the mouth of the Kalambo river, a tributary of Lake Tanganyika, numbers of *Gl. palpalis* were seen to settle at the edges of little puddles of stagnant water containing various low forms of plant life. We could not determine that they were sucking up the water, and at the time were not in a position to examine the flies.

Nine of the 113 *Gl. morsitans* were found to be infected with protozoa, i.e., 7.8 per cent. This intestinal infection apparently tends to die out, or at all events, the parasites do not retain the ordinary trypanosome form after a few days, as is evidenced by the following:—

Time of examination	No. EXAMINED		
	Male	Female	Percentage
Freshly caught ...	31 (5 positive)	2 (negative)	15.1
24 hours after capture ...	60 (4 " )	6 "	6
48 " " "	2 (negative)	0	0
96 " " "	12 "	0	0

## V. INCIDENCE OF INFECTION IN PROBOSCIS

As stated above, infection of the proboscis of *Gl. palpalis* with the parasites was never observed. It was found to be present in seven out of thirty-one *Gl. morsitans*, 21.2 per cent. In contradistinction to the intestinal infection, that of the proboscis apparently increases in frequency on keeping the flies, e.g.:—

Time of examination	No. examined	No. infected	Percentage	Condition of intestine in infected cases
Freshly caught ...	8	1	12.4	Parasites present
24 hours after capture	10	4	40	Parasites present in 3, absent in 1
48 " " "	3	2	66.6	Parasites present in 1, ? in other, fly lost
96 " " "	12	0	0	



Taken in conjunction with the figures given above, this may indicate, as Stuhlmann<sup>5</sup> suggests, that the intestinal infection gradually works forward, and that the proboscis is infected last. If the intestinal forms really die out in two or three days, the reinfection might be explained by some of the proboscis forms, which are almost always present in the hypopharynx, being ingested with a succeeding feed.

#### VI. MORPHOLOGY OF FORMS FOUND IN *GL. PALPALIS*

In fresh preparations, as in stained ones, the parasites presented a great diversity of size and shape, from extremely long narrow forms to short broad ones. One variety had a body somewhat like the section of a bi-convex lens, with a long, lash-like flagellum; a second variety was a very narrow, rod-like organism, which moved with considerable rapidity; a third variety was 'beaked,' that is, the posterior portion was narrow, and succeeded by a more or less central bulbous portion which tailed off into the flagellum. The development of the undulating membrane varied from nothing, to one which was fairly-well defined. The two types, long thin ones and short clubbed ones, could almost always be seen, though, of course, many intermediate forms between these two were also present. In those flies in which the parasites were few in number, they were as a rule of the broad short type. In the majority of cases the trypanosomes were extremely numerous, and in a few instances large clumps of them were seen adhering by their flagellate extremities to the gut wall towards the distal end of the intestine, similar to the clumps described by Novy, McNeal and Torrey<sup>12</sup> in mosquitoes. The trypanosomes composing these clumps moved slowly from side to side. In addition to the free forms carrying flagella, rounded motionless parasites devoid of flagella were also observed in fresh preparations. These are apparently derived from the 'broad' type of organisms.

In only one fly were the parasites observed outside the true intestine. In the one referred to, a few were seen in the preparation from the proctodaeum. This fly had been fed on a dog about an hour previously.

Trypanosomes, or any bodies recognisable as derived from them, were never seen in the salivary glands, malpighian tubules, testes, fat bodies, ovaries, or other organs of the flies.

*Stained preparations.*

The description is given almost entirely from the slides which were examined in Africa. Like Minchin, we have found that the ones which were left to be stained at home have proved to be practically useless.

In general, the trypanosomes may be divided into the two types, long narrow ones, and broad shorter ones, which have been mentioned above.

The long type of organism is chiefly met with in two separate forms. The first of these measures from  $30\mu$  to  $40\mu$  in length by  $1.7\mu$  to  $2\mu$  in width. The posterior extremity is usually rounded or bluntly angular, while the anterior, or flagellar extremity, is more or less acutely drawn out. The body width is uniform throughout the greater part of the length of the parasite, so that it bears some resemblance to a narrow strip of ribbon. The nucleus is oval; as a rule occupies the whole width of the body, and does not present any peculiarities of structure other than in a few instances; being composed of a central, deeply-staining portion, from which projects at either pole a more loosely-built wing. The blepharoplast is large, oval or round, and is usually placed in close apposition to the nucleus. More generally its position is anterior to the nucleus, but it may be lateral or posterior, and in some instances is separated by an appreciable interval from it. The undulating membrane, as a rule, is not well marked, and may not be present. The flagellum varies from examples in which a distinctly free portion of some length is present, to those in which none is discernible. A slight club-shaped thickening of the terminal portion of the free flagellum is not uncommon, and in one or two cases the root is expanded into a fan-like arrangement close to the blepharoplast. The cytoplasm stains a darkish blue (Giemsa's stain), and may contain a few small granules, though commonly it is free from these, and is quite homogeneous in composition.

The second variety of the long forms is an extremely narrow and elongated one, which may measure as much as  $53\mu$  in length by only  $0.5\mu$  in width. The posterior extremity is acute, while the anterior end is drawn out very gradually along the flagellum. The nucleus is long, and occupies the whole width of the body. No definite

structure could be made out in it. The blepharoplast is rounded, and is usually superimposed on the nucleus. There is no visible undulating membrane, and the flagellum is prolonged into a well-defined free portion. The protoplasm stains a light blue, and is free from granulations.

This type of parasite is not nearly so frequent as the one previously described. They were seen in preparations made from a fly which had fed forty-eight hours beforehand on a hen, and from one which had fed an hour earlier on a monkey, but in this case they were not so long as the ones from the hen-fed fly. The broad forms of the organisms were also present in these preparations.

The more usual form of the broad type approaches in shape that of the ordinary blood trypanosomes. They measure from  $19\mu$  to  $30\mu$  in length and  $1.8\mu$  to  $3\mu$  in breadth. The posterior extremity is more or less rounded; the anterior end is attenuate. The nucleus is oval, and stains homogeneously. The blepharoplast is rounded or oval and occupies much the same position in relation to the nucleus as in the long forms. The undulating membrane is usually perceptible. The flagellum does not show the tendency to become clubbed, which was noted in the other variety. The protoplasm stains a light blue or pink, and may contain some granules.

Various modifications of the broad type exist, and the 'beaked' kind is probably one of them. In this form, the posterior extremity instead of being rounded in the ordinary manner, is elongated, so that the expanded nuclear area occupies a central position between two attenuated portions. The posterior extremity is not, however, extended into a filiform portion like the anterior end, but is usually cut off squarely. In other respects it is much like the ordinary broad type.

In most of the preparations, oval or rounded forms measuring about  $4\mu$  or  $5\mu$  in diameter, and without a flagellum, were present. The protoplasm stains darkly, and may contain granules. These forms are apparently derived from the ordinary broad ones. The posterior portion of the parasites containing the nucleus and blepharoplast become globular, while the anterior part loses its staining powers, becomes irregular in shape, and degenerates. The flagellum disappears coincidentally. No definite retaining wall is present, so that these forms are not true cysts. Encystment, as described by Minchin,<sup>7</sup> has not been observed in our preparations.



While the formation of the rounded forms may be traced, the reverse process, development into flagellated parasites, may also be seen from bodies very similar to *Leishmania*. The flagellum appears first as a comparatively short structure, and gradually increases in length, the body of the trypanosome becoming stretched out at the same time to assume the more ordinary character.

Division was seen in organisms of the broad type alone. Ordinary simple fission is the commonest, but instances occur in which a triple division is present.

Many gradations between the long and broad forms are present; probably immature specimens of these types. The appearances of all the forms agree with the description of *Trypanosoma grayi*, Novy, and to this category we would refer the parasites we have seen in *Glossina palpalis*. No forms recalling *T. tullochii* were observed.

#### VII. MORPHOLOGY OF FORMS FOUND IN *GLOSSINA MORSITANS*

##### A. IN INTESTINE. *Fresh examination.*

(1) Long filiform forms, of approximately the same width throughout the greater part of the body, but tapering gradually towards the flagellar extremity.

(2) 'Beaked' forms, with a narrow posterior portion, succeeded by a more or less oval central mass, and then tapering gently towards the anterior end.

(3) Blunt or clubbed forms, with a rounded posterior portion, which is the widest part of the body.

(4) Regularly oval or ovoid forms, with a flagellum apparently devoid of any undulating membrane, projecting from the narrow pole.

(5) Motionless oval or rounded forms devoid of flagellum.

The first four varieties are motile. The 'beaked' forms are rather peculiar, in that the posterior and bulbar portions always remain quite rigid, while the anterior portion is vibratile. This is explained by the position of the blepharoplast close to, and ordinarily in front of the nucleus, which lies in the expanded middle part of the parasite.



In all cases the parasites were much more abundant in the posterior part of the midgut. They were never observed outside the intestinal tract, and never in the proctodaeum.

*Stained preparations.*

The long forms measure from  $26\mu$  to  $35\mu$  in length and from  $1.8\mu$  to  $2\mu$  in width. Both ends are more or less acute, but the anterior is the more attenuated of the two. The nucleus is about  $4\mu$  to  $4.5\mu$  in length, is oval, and usually occupies the whole width of the body. The blepharoplast is relatively small, and in contradistinction to the forms seen in *Glossina palpalis*, is usually posterior to the nucleus, and is separated by an appreciable interval from it. The undulating membrane is very poorly developed, and the flagellum does not, as a rule, attain to any great length. The general protoplasm stains a rather deep blue, and is homogeneous in structure.

The clubbed variety measures  $20\mu$  to  $25\mu$  in length by about  $3\mu$  in width. The posterior extremity is rounded, and the body attains its maximum width a short distance anterior to this point. From the point of greatest width it tapers gently to the anterior end. The nucleus is rounded or oval, and is situated more towards the posterior part of the body. The blepharoplast, in these forms, frequently is placed anteriorly to the nucleus, and is usually small. The flagellum and undulating membrane are not well marked, and the free part of the flagellum is quite short. The body protoplasm stains rather lightly.

The 'beaked' forms are of comparatively frequent occurrence, but do not present any other peculiarity of structure than the special shape of the posterior part of the body. This has a rounded end, and is very narrow until it approaches the nuclear area when the body expands into an oval portion, beyond which it becomes gradually drawn out towards the anterior end.

The oval, flagellated forms when stained are seen to be composed of a large globular mass, from one end of which the body is prolonged as a narrow process containing the flagellum. The enlarged portion measures from  $7\mu$  to  $15\mu$  in length by  $4.5\mu$  to  $6\mu$  in width, and the prolonged portion from  $6\mu$  to  $12\mu$ . The nucleus is usually oval, and stains uniformly. The blepharoplast is rounded, and is usually in

close connection with the nucleus, either slightly anterior to or superimposed on it. No definite undulating membrane is present, and the flagellum is co-terminous with the prolonged portion of the parasite. The protoplasm may contain a few granules, and usually stains a uniform and rather deep blue.

The oval or rounded motionless forms are, except that they are devoid of flagellum, practically identical with the ones just described, which are apparently derived from them by the growth of the flagellum and coincident lengthening of the body. As in the case of similar forms seen in *Glossina palpalis*, no trace of a cyst wall is present.

Division forms are present amongst parasites of the broad type.

The parasites in the intestine of *Glossina morsitans* recall closely those of *Glossina palpalis*, the only marked difference being the almost uniformly posterior position of the blepharoplast, and the greater tendency to acuity in the posterior part of the body. These differences are not sufficiently decided, and the amount of work we were able to devote to the study is too small to enable us to say that they are a different species.

#### B. IN PROBOSCIS.

##### *Fresh preparations.*

In the proboscis of freshly-caught flies the parasites were found, as noted by Roubaud,<sup>9</sup> adhering to the labium and labrum in large clumps, and occasionally so numerous as apparently to occlude the lumen. Moreover, we observed them frequently within the hypopharynx also, in extremely large numbers. In both situations, the clumps, which resembled a chrysanthemum, were formed of very many parasites which had the flagellar extremity directed towards the walls of the proboscis. They moved with a slow wave-like action, and also by contraction. Single, separate organisms were fairly active, and, as described by Roubaud frequently became attached to the slide or to bits of tissue in the preparation. As a rule, they were present throughout the whole length of the proboscis, though variations were noticed from a decided massing of the parasites at the distal extremity to a scanty occurrence at the proximal end.

The parasites, in the fresh, appeared to be of various sizes and shapes. Some were almost filamentous, others were attenuated at either extremity, at the flagellar end more markedly so, while others again approached more closely the ordinary shape of the pathogenic trypanosomes. They appeared to be about one and a half times the diameter of the proboscis in length.

Parasites were generally present in the gut at the same time as in the proboscis, although this was not invariably the case. In at least one, no trypanosomes were seen in the intestine.

#### *Stained preparations.*

A special description of the forms seen in the proboscis is unnecessary, as they correspond closely to those observed in the gut. In general their dimensions are less, but in other respects the description of the various intestinal types applies equally well to those of the proboscis.

The beaked variety was the commonest, and after that the ordinary broad club-like forms. The long ribbon-like type was not seen in stained preparations, though a few forms resembling on a small scale the very narrow long type were observed. In many of the forms the flagellum did not stain at all, and, if present, was extremely rudimentary.

### VIII. FEEDING EXPERIMENTS

These were originally undertaken in order to trace, if possible, a development of the 'wild' trypanosomes in *Glossina palpalis*. Many different sources of food were used, mammalian and avian, and the

Source of food	Flies fed	Flies examined	Positive	Present
Dog ... ..	6	4	0	0
Monkey ... ..	42	38	4	10.5
Guinea-pig ... ..	2	2	0	0
Sheep ... ..	20	19	3	15.7
Hen ... ..	17	17	2	11.6
Hornbill ... ..	3	3	1	33.3
Darter ... ..	1	1	1	100
Small, canary-like bird ...	1	1	1	100

11.1 %

22.7 %



intestinal contents of the flies were examined at various periods afterwards, from a few hours to several days. The results of this examination were rather surprising, for whereas 42 per cent. of unfed (artificially) flies contained flagellates, only a very small proportion of the fed ones were found to harbour parasites.

The discrepancies shown by these figures are rather interesting, and rather difficult to explain, unless it be due to the fact that mammalian blood exerts some unfavourable action on the parasites in these flies. As we have already said, mammalian blood does not form any part of the ordinary food-supply of the flies on the island. The difference in the results between the flies fed on the hen and those fed on birds inhabiting the island is also suggestive. However, the results are so scanty that no great weight can be laid on the suggestion we have offered.

In Uganda, Minchin, Gray and Tulloch<sup>3</sup> found that goats' serum acted deleteriously on the 'wild' parasites, and found that by this means they could be distinguished from *T. gambiense*. Other sorts of serum had, however, no such action.

The following experiment was made to ascertain whether crocodile serum was more favourable to the parasites than that of some of the local birds.

Hanging drop preparations were made from the intestinal contents of a fly containing very many parasites, and were diluted in the proportion of 1 : 1 with salt-citrate solution and serum obtained from a crocodile, a hen and a darter. The preparations were examined at intervals of fifteen minutes for a period of four hours. The changes which occurred, consisted of a clumping of the parasites with the flagella towards the periphery, and a gradual cessation of the movement of the clumped forms. Simultaneously they started to break down, until finally there was left a granular and refractile mass. The clumping occurred rather more slowly in the preparation containing crocodile serum than in the other two, but otherwise precisely similar changes occurred, and at the end of four hours there were only a few free trypanosomes left.

A few *Glossina morsitans* were fed on a dog. In one series ten flies were fed, and examined at intervals of from one and a quarter to three hours later. None of them contained parasites. In another series eleven were fed on the same dog, and examined forty-eight



hours later. Two of these flies contained intestinal parasites. In one they occurred as small clumps with the flagellar ends directed towards the periphery, and in the other as long freely motile forms. Parasites were also present in the proboscis of this latter fly.

#### IX. ORIGIN OF PARASITES

Some conflict of opinion exists as to the origin of these 'wild' parasites. It would seem that, to a certain degree, this may be explained by the views held by various writers on the question as to the food of tsetse flies. Novy,<sup>4</sup> who thinks that they may possibly feed on other substances than blood, is of the opinion that the parasites may possibly be derived from stagnant water, or some similar fluid. Reference has already been made to an instance in which the flies may have been imbibing such a fluid. Against this, however, is the experience in raising and maintaining tsetse flies in captivity, which, so far, has shown that blood is the only food which they will ingest.

On the other hand, Minchin<sup>7</sup> and others consider that blood alone is the natural food of the tsetse flies, and that in all probability the parasites are derived from a vertebrate host, possibly a bird. To a certain extent, the infection may be due to the 'contaminative' transmission, that is the ingestion of the cystic forms of *T. grayi*, though the probability of the continuation of the fly-infection in this way is rather slight.

So far, no vertebrate host of these parasites has been discovered. On the island we examined the blood of darters, pigeons, hornbills, several species of small birds, snakes, mice and crocodiles (4), but in none of them were parasites of any description seen. *T. sp.* (?) were seen in frogs caught on the mainland.

The difference of opinion on the question of hereditary transmission also tends to complicate the solution. Patton and Strickland,<sup>6</sup> who incline to this hypothesis, quote in support of their view the instance in which Minchin, Gray and Tulloch<sup>3</sup> observed the infection in a laboratory raised fly which had been fed on a hen. If hereditary transmission of the flagellates, and food other than blood are eliminated, it is rather difficult to account for the infection of so large a percentage of *Gl. palpalis* on the island. It may be

argued that if hereditary transmission does occur, all the flies in such a restricted area should have been infected. As is known, the infection tends to die out of the intestine, or at least apparently so, though the persistence of unrecognised forms of the parasites cannot yet be excluded. This, however, depends on the lack of suitable pabulum, and it is within the bounds of possibility that when a 'negative' fly does get a meal of the required sort, the ordinary forms of the flagellates may be evolved from the unrecognised forms. In the flies we examined, traces of a meal (blood) were found in 33.1 per cent. and parasites in 42 per cent., but as the blood is digested in a shorter period of time than the parasites take to disappear from the intestine, this discrepancy can be accounted for. To some degree, the reinfection of the intestine may be due to ingestion of the proboscidal forms, provided that the fly feeds a second time before these have died out.

The further point also arises as to the identity of the forms seen in *Gl. morsitans*. In general they appear to be similar to those found in *Gl. palpalis*, except in minor points. If they are the same species, many species of vertebrates must harbour the parasites, if we accept the view that they are derived from vertebrates, since *Gl. morsitans* feeds to a great degree on a different fauna from that on which *Gl. palpalis* feeds. There is no reason to suppose that the two species are identical other than the similarity in form, and this is not a decisive feature for the determination of species amongst the trypanosomes.

Diversity of opinion also exists as to the origin of the forms found in the proboscis. Stuhlmann<sup>5</sup> considers that they are derived from the intestinal forms, the infection of the proboscis being the final stage in the development of ingested trypanosomes. Roubaud,<sup>9</sup> on the other hand, thinks that the infection in the proboscis is an entirely distinct thing and that it is to be accounted for by the fixation and multiplication of pathogenic trypanosomes, *in situ*, which have been ingested in the act of feeding.

There are several points which tend to invalidate Roubaud's conclusions. He does not state whether he examined the proboscides of freshly-caught, unfed flies for the presence of flagellates, and it is possible that he mistook the naturally-occurring forms for those

developed from pathogenic ones. If they are derived from these, and not from the ordinary intestinal forms, there does not seem to be any reason why the development in the proboscis should only occur in 10 per cent. of the flies fed, and not in all, and this is the percentage in which intestinal infection was present in his flies. Moreover, he does not give any reason for stating that the intestinal parasites are cultural forms of *T. pecaui* which must have been derived from big game. In the first place, it is extremely rare to find trypanosomes in buck, and, secondly, it does not follow that such parasites as may be present are *T. pecaui*. We do not wish to criticise unnecessarily, but in working with tsetse flies great caution must be observed before reaching definite conclusions. The greatest objection to the assumption that the forms seen in the proboscis are derived from pathogenic trypanosomes is that they are innocuous when injected into susceptible animals.

Our results are discordant, for in *Gl. morsitans*, both intestinal and proboscidal forms were encountered, while in *Gl. palpalis* only the first were seen. This may be accidental, and more continued work might have resulted positively.

Of the two hypotheses we are inclined to agree with that of Stuhlmann: that the parasites in the proboscis are derived from those in the intestine.

It is, perhaps, possible that the parasites occurring in 'wild' tsetse flies, as distinct from laboratory bred ones, may be derived from pathogenic trypanosomes which lose for some unknown reason their infectivity when ingested, and it is possible that the intestinal forms do not all belong to one species but represent a mixed infection. This can only be decided by lengthy experiments.

In the case of *Gl. morsitans* it would seem on *a priori* grounds that a development of the pathogenic trypanosomes does occur, for it is a reasonable certainty that any susceptible animal exposed to their bites in nature will become infected. All such infections cannot be explained by a mechanical transmission. The recent work of Kleine, and its confirmation by Bruce, shows that a development apparently does occur.



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# SECOND REPORT ON HUMAN TRY- PANOSOMIASIS IN NORTH-EASTERN RHODESIA AND NYASALAND

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	SYNOPSIS	PAGE
I.	Introduction .....	278
II.	Physical characters of country in relation to occurrence of tsetse flies .....	279
III.	Distribution of tsetse flies	
	1. <i>Glossina palpalis</i> .	
	(A) In North-Eastern Rhodesia .....	281
	(B) In Nyasaland .....	283
	2. <i>Glossina morsitans</i> .	
	(A) In North-Eastern Rhodesia .....	283
	(B) In Nyasaland .....	283
	(C) In North-Western Rhodesia .....	284
	(D) In the Katanga (Belgian Congo) .....	284
	3. <i>Glossina fusca</i> .	
	(A) In North-Eastern Rhodesia .....	284
	(B) In Nyasaland .....	284
IV.	Habits of <i>Glossina palpalis</i> .....	284
V.	Habits of <i>Glossina morsitans</i> .....	287
VI.	Gland palpation and puncture .....	291
VII.	Occurrence of cases of Trypanosomiasis.....	300
VIII.	Mode of introduction into country .....	301
IX.	Prophylaxis .....	302

## I. INTRODUCTION

In our previous report,\* published in June, 1908, we reviewed shortly the conditions we had found in North-Eastern Rhodesia, mentioned the distribution of tsetse flies so far as we had ascertained it at the time, and made several suggestions as to measures designed to limit the further extension of human trypanosomiasis. Unfortunately, a second report despatched in October, 1908, went astray, so that some of the observations embodied in the present paper are rather belated. It has been deemed advisable, however, to incorporate them for the sake of completeness.

On leaving Madona, on the Luapula river, in February, 1908, we proceeded to Lake Bangweulu and after spending some days on its western shores and on its islands, separated; one of us now covering the country drained by the Chambesi river, while the other followed the Kalungwisi and Luangwa rivers to Lake Mweru, thence along the northern Anglo-Congolese boundary and the British section of Lake Tanganyika. This work was completed at Abercorn in June, 1908. The next few months were spent, partially on an island at the south end of Lake Tanganyika, and partially at a camp established about thirty miles from this lake, on the bluff overlooking the Lovu river. We left for England on the 15th of November, 1908. One of us proceeded straight from Abercorn to railhead—Broken Hill,—the other travelled across the Tanganyika-Nyasa plateau to Karonga, and then by way of Lake Nyasa and the rivers Shiré and Zambesi to Chinde. The expedition reached England on the 27th of March, 1909, after an absence of twenty-three months, some twenty of which were actually spent in the country. The routes followed, together with the distribution of tsetse flies in North-Eastern Rhodesia, Nyasaland, and portions of North-Western Rhodesia and the Congo Free State, will be found on the attached map. Some seven thousand miles, exclusive of small trips, have been covered, and observations have been made over a large portion of these countries, but it should be noted that practically all the places were visited only once, so that no very great degree of finality exists in the work. In a few instances we were able to institute comparisons at different seasons of the year.

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\* Kinghorn and Montgomery, 1908. *Annals Trop. Medicine and Parasitology*, Vol. II, No. 2.

With the exception of the distribution of tsetse flies, most of our information is first-hand, as until Europeans started to search for cases of Sleeping Sickness, the natives were in entire ignorance of the disease, and even yet have not grasped the danger which attaches to it. In many parts of the country they denied the presence of tsetse flies, although these were caught within the village.

For instance, at Kundushia's, on the Mansa river, the headman said that there were no tsetse flies (*tusembe*) in the neighbourhood. Three specimens of *Gl. morsitans* were caught in the village.

At Komba's village, on Lake Tanganyika, we were told that there were no tsetse flies. *Gl. palpalis* was fairly abundant along the lake shore.

This was due, in all probability, to the fact that they did not possess any live stock, for in those districts where domestic animals were found, the owners knew the flies and the disease which they produced.

## II. PHYSICAL CHARACTERS OF THE COUNTRY

The general consensus of opinion is that *Glossina palpalis* does not exist at elevations above four thousand feet, and although Dutton and Todd\* mention an instance in which they were found above this height, reported on the authority of an officer in the Congo Free State service, confirmatory observations are lacking. In view of this, it is of some interest to take notice of the physical geography of North-Eastern Rhodesia and Nyasaland, since the greater part of these two countries is formed of high plateau ground. Speaking broadly, North-Eastern Rhodesia may be described as composing a part of the watershed, running diagonally south and west, which separates the Congo and Zambesi systems, and which, for the most part, reaches an elevation of from four to over five thousand feet above sea level. It is split up into more or less parallel sections by the Luapula, Chambesi and Luangwa rivers, and in many places the fall from the plateau to these river valleys is very abrupt, as, for example, on the west side of the Luangwa where the Machinga escarpment rises very acutely some two thousand feet. This abrupt fall is particularly

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\* Dutton and Todd, 1907. *Annals Tropical Medicine and Parasitology*, Vol. I, No. 1.

Martin (*Les trypanosomes de la Guinée française*, 1906, A. Maloine, Paris) mentions that he caught a single specimen of *Gl. palpalis* at an elevation of 1,800 metres.



marked at the south end of Lake Tanganyika and the north end of Lake Nyasa, where, in both cases, it occurs almost at the water's edge. At the south end of Lake Nyasa and in the neighbourhood of Lakes Mweru and Bangweulu, the slope is more gradual, and the level of the plateau is only reached at a distance of forty or fifty miles from the river.

Neave\* has reported that the occurrence of *Glossina palpalis* on several of the rivers in the Katanga, stops rather suddenly about  $10^{\circ}30'$  S., in some cases coinciding with the abrupt rise to the plateau level. On the Luapula river no such marked transition occurs; indeed the drop between Lake Bangweulu and Lake Mweru is only some seven hundred feet (3,700-3,000 ft.), but the stoppage of the fly at about  $11^{\circ}30'$  S. corresponds, more or less, with a change in the vegetation of the river banks. North of that latitude, the banks are fairly well wooded, while south of it, the river runs for long stretches through bare, flat country, and this probably has acted as a bar to the extension of *Gl. palpalis* to Lake Bangweulu and the Chambesi Valley.

Neave also suggests that the absence of *Gl. palpalis* from the greater part of Rhodesia and Nyasaland may be due to the change in the fauna, which occurs in this portion of Africa, from the West Coast type to that of the Eastern and Angolan regions. Apparently this species of fly oversteps the boundary, for it was captured on the Zambesi, between Tete and the Victoria Falls, by Sir John Kirk.† Since the conditions on Lake Nyasa, on the Luangwa river and on other tributaries of the Zambesi are quite favourable for the existence of *Gl. palpalis*, it is very important to ascertain as soon as possible whether it is still to be found anywhere along this system of waters.

Altitude is unfavourable to the development of *Gl. palpalis*, probably on account of the temperature. According to Roubaud‡ the most suitable temperature varies between  $25^{\circ}$  and  $30^{\circ}$  C., and on the plateau the mean is lower than this. At Kambole, on the Tanganyika plateau (about 4,800 feet above sea level) where careful records were kept for some years, the mean daily temperature was found to be  $21^{\circ}$  C., and the variation between  $7.2^{\circ}$  and  $32.2^{\circ}$  C. These conditions

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\* Neave, 1908. Report of Katanga Medical Commission, London.

† Austen, 1903. Monograph of the Tsetse flies.

‡ Roubaud, 1908. Bulletin Soc. Pathologie exotique, T. I, No. 5.



would hold good for many portions of the country, and it may safely be said that on the plateau the vegetation and temperature approach the temperate more closely than the tropical type. At the level of the lakes and in the large river valleys, the conditions, of course, are much different, and are sufficiently well marked to be quite perceptible in passing from the low to the high levels.

There are other factors, than the altitude and temperature, which would tend to limit the presence of *Gl. palpalis* in the main portion of the territory with which we are dealing. The annual rainfall, for instance, is comparatively low, from 40 to 50 inches, and the drainage is so complete that, except in the rains (end of October to end of March), all but the main rivers are dried up, so that the air lacks the humidity which is apparently necessary for the development of this species. The lack of shade is another factor. The whole of the country is covered with a very thin type of bush formed by small, scraggy trees which afford very little shade. At frequent intervals the bush is interrupted by grassy 'dambos' or plains, of varying size, which, in the rains, act as reservoirs into which the water drains from the surrounding localities and from which the streams take origin.

The shores of Lake Tanganyika are anomalous, in that the fly is found where there is practically no shade, but the greater humidity and higher temperature are doubtless important factors in determining their presence there.

Most of the streams in Rhodesia and Nyasaland have been insufficiently explored for *Gl. palpalis*, and while it has not as yet been found on many of them, it would be advisable that more time should be devoted to the search before an absolutely negative result is recorded. So far as the general conditions go, there is no reason to suppose that the species would not flourish just as well on many of the more central streams as they do, for example, on parts of the Luapula.

### III. DISTRIBUTION OF TSETSE FLIES

#### 1. *Glossina palpalis*.

##### A. In North-Eastern Rhodesia.

In our earlier report, we said that this fly had been found on both banks of the Luapula river from Kapwepwi's (11°30' S.) to Kasiwa's, a village on the British side about 10°15' S. Beyond Kasiwa's,

proceeding downstream, the river is bordered by gradually widening, swampy flats, along which the fly has not been found.

We went along the British bank of this portion of the Luapula river in January, at the height of the rains, but could not travel by water as there were no canoes available. Dr. Spillane covered it in October, and reported that he could not find *Gl. palpalis* at that time (commencement of rains).

On the Belgian side, however, the banks are high and protected by trees, and it is quite probable that the species exists farther down stream on this side of the river although we cannot speak definitely on this point.

The fly is also found on the Kalungwisi river, along a small stretch, about ten miles in length, starting just below the old Kalungwisi station and proceeding up-stream. Here the river flows through low hills which are well-wooded to the water's edge. Above these hills to the point of emergence from the Machinga (the hills at the edge of the plateau) and, again, below Kalungwisi boma to Lake Mweru, the river runs between low, grass-covered banks, bounded on either side by wide, marshy flats, and along these portions of its course *Gl. palpalis* has not been found.

On two separate occasions, in May, 1908, just at the cessation of the rains, we explored the river by canoe, but saw no trace of *Gl. palpalis*.

In May, 1908, we followed the Kalungwisi to its junction with the Luangwa river, and then went up this stream for some distance, but were unable to find any trace of the fly, although in many places the conditions were most favourable. In its upper reaches, the Kalungwisi, like so many of the Rhodesian rivers, flows through very wide grassy plains almost completely destitute of cover.

On Lake Mweru.—The fly is not uniformly distributed around the whole shore of this lake, but occurs in small patches where the trees and bushes approach close to the water. On the open sandy beaches it is absent. It is also found for some distance up some of the tributaries of the lake, for example the Luao and the Luchinda, and of course along the Lualaba after its exit from the lake. It is also present on the island Kilwa (Spillane).

On Lake Tanganyika.—*Glossina palpalis* occurs around the whole extent of the British shore, and up the principal confluent of this part of the lake, the Lovu river, for a distance of from fifty to sixty miles from its mouth (i.e., a short distance above the junction of the

Mukotwe river). Above this point, the change in the character of the country probably accounts for its absence. The fly is also found for shorter distances up some of the smaller tributaries of the lake and the Lovu.

The fly was first found on Lake Tanganyika by Spillane in November, 1907 (beginning of the rains). We found it on the Lovu in June, 1908 (dry season), and also at many places on the lake. In July and August, 1908, the fly was traced up the Lovu river by Spillane and S. A. Neave. We utilised canoes in this work.

During the months of March and April, 1908, some time was spent on the shores of Lake Bangweulu and on its islands, as well as along some of its confluent, but on none of these were we able to find *Gl. palpalis*. The western shore of the lake and some portions of the islands have definite, wooded banks, but the northern and eastern sides have not. On these sides, all the tributaries of the lake flow through large swampy plains as they approach it, and these, in turn, expand into immense marshes around the open water. The Chambesi river with some of its affluents (April and May, 1908), the Luapula from Lake Bangweulu to Chongola's village (September, 1907, and March, 1908), and a portion of the Luangwa flowing into the Zambesi (August, 1907), were examined with negative results. These have also been searched by S. A. Neave with the same result.

#### B. In Nyasaland.

*Gl. palpalis* has not yet been found on the lake or any of its tributaries. The only portions we have examined are the northern end of the lake, and parts of the Songwe, Lufira, and North Rukuru rivers, in December, 1908.

### 2. *Glossina morsitans*.

#### A. In North-Eastern Rhodesia.

In this country *Gl. morsitans* has an extremely wide distribution.

The only parts which can be said, with any degree of certainty, to be free, are the Tanganyika-Nyasa plateau, the plateau in the neighbourhood of Serenje, and a small district around Fort Jameson.

#### B. In Nyasaland.

The fly is reported\* as being absent in the mountainous northern districts of the country. Its chief distribution is along the low

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\* Survey made by the late Capt. F. H. Hardy, R.A.M.C., and Dr. Wykesmith.



country between the lake shore and the hills, starting about 12° S., and extending down the Shiré river for some distance.

C. In North-Western Rhodesia.

The distribution has been given in our earlier report,\* and includes most of the country north of the hook of the Kafue river.

D. In the Katanga district of the Belgian Congo.

According to Massey,† *Gl. morsitans* is present over most of this territory, and we have established the continuity of its area of distribution in the Katanga with the bounding areas in N.W. and N.E. Rhodesia.

3. *Glossina fusca*.

A. In North-Eastern Rhodesia.

A specimen of this species is reported‡ to have been caught on the Luangwa river close to its junction with the Zambesi.

B. In Nyasaland.

It has been caught on the road running past the elephant marsh, near Chiromo, and also near Kaporo's village on Lake Nyasa, to the north of Karonga. It is not very common at either of these places, and only one or two specimens have ever been caught at a time. We failed to find it near Karonga in December last.

#### IV. HABITS OF *GLOSSINA PALPALIS*

Our observations accord, in most particulars, with those which have been given by other workers. On many occasions we were struck by the apparent indifference these flies displayed in the presence of food, and although they would alight on the bare skin of a native, they would often remain quiescent for a considerable length of time without making any effort to feed. In this respect they differ most markedly from *Gl. morsitans*, for this species, as a rule, does not

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\* Montgomery and Kinghorn, 1908. *Annals Trop. Med. and Parasit.*, Vol. II, No. 2.

† Private communication.

‡ Private communication, Administrator of N. E. Rhodesia.



waste any time, but proceeds to fill itself voraciously almost as soon as it has settled. So far as our experience goes, *Gl. morsitans* is much the more troublesome.

On the march, wherever *Gl. morsitans* was at all plentiful, all the carriers provided themselves with bunches of leaves, and the constant sound of these striking the skin bore evidence to the viciousness of the flies.

In most places the conditions under which we caught *Gl. palpalis*, approach closely to those obtaining in Uganda and other parts of Africa; that is, there was fairly abundant shade afforded by the trees and bushes along the water courses, but on Lake Tanganyika a most striking difference was noticed. This lake is closed in by precipitous cliffs from one to two thousand feet high, which come to within a short distance of the water. The shores shelve gradually from the foot of the hills, are sandy or pebbly, and are either quite devoid of vegetation, or, at most, furnished with small patches of the reed known locally as 'mtete' or 'bango' (*Phragmites communis*). In only a very few places does this assume any luxuriance of growth, yet it was noted that the fly could almost always be found at even the scantiest collection of isolated reeds. They were frequently seen basking in the sun, on the rocks at the water's edge. The whole picture presented by these rocky beaches with small, scattered patches of reeds differs very materially from what has been considered to be typical '*palpalis*' country, i.e., wooded banks affording plentiful shade. This may be a local peculiarity of the species (*Glossina palpalis*, Rob.-Desv., var. *wellmani*, Austen, and intermediate forms between this and the type species), or it may indicate that in the absence of more favourable conditions, other factors being suitable, the fly is capable of a certain amount of adaptability, and this, of course, becomes of some importance in considering the possible extension of the species. We have been informed by Prof. Todd that *Gl. palpalis* is common on long stretches of the Upper Congo, where the banks on either side are covered with grass alone.

On the Lovu river, along the portion examined by us, the banks are covered with a dense growth of the reed we have mentioned above, and only an odd bush or two, yet the fly was very abundant. The shade afforded by the reeds was, however, very deep here.

Many observers have noted a seasonal variation in the number of

the flies found, and, curiously, the one or two instances of this which we have seen, have been the reverse of the usual statement that they are more abundant in the wet season.

The Lovu river was visited by Dr. Spillane, the P.M.O. of N.E. Rhodesia, in November, 1907, when the rains were on, and at that time no flies were seen by him; yet when we got there in June, 1908, two months after the close of the wet season, they were particularly plentiful. It is not clear why this should be so, for along this particular stretch of the river there are many villages and an assured supply of food. On an island in Lake Tanganyika, which was visited at the height of the dry season (July), and again at the commencement of the rains (November), there was no apparent difference in their numbers, but these were so great that it would be difficult to draw any definite conclusions. On the lake shore, however, fly were caught during the rains at several places where they were not seen in the dry season.

There is apparently a seasonal variation in the proportion of the sexes. On the island referred to, of 1,409 specimens of *Gl. palpalis* caught in July (height of dry season), 1,282 were males and 127 females, a proportion of 10 : 1. In November, just after the rains had set in, of 401 specimens caught at the same place, 396 were males and only five females, a proportion of 79 : 1. This may indicate that it is at this time of the year that the females are depositing larvae, and that during this process they become less active than in the dry season, when the humidity and the temperature are lower.

In spite of prolonged and careful search, we were never successful in finding pupae, and in this our experience coincides with that of Ensor\* in the Soudan. It may have been due to the fact that the island was composed of very rough, broken stone, with very little soil, so that the pupae might easily have dropped into the crevices of the rocks where they could not be found.

Our search was made along the shores of an island, just above the edge of the water and two or three yards inland. We were assisted by five or six 'fly-boys' trained in the work, and although they often brought us pupa cases they were never those of *Gl. palpalis*. On each occasion, several hours were devoted to the work, and during our stay on the island, in the months of July and November, we searched at least half a dozen times. We always selected the places where the shade was most abundant.

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\* Ensor, 1908. Reports of the Wellcome Research Laboratory, No. 3.

It is hardly necessary to consider at any length the question as to whether crocodiles form the staple article of diet of *Glossina palpalis*, as has been suggested by Koch.\* In captivity the fly will feed on various animals—we have used dogs, monkeys, sheep, goats, hens, a hornbill, and some small birds—and in its wild state the source of food will be determined altogether by the local fauna. In some places where *Gl. palpalis* exists, there are no crocodiles; so that the association of the two is more or less accidental.

For instance, at Madona we were informed by residents of many years' standing that it was an extremely rare event to see a crocodile, yet the fly was plentiful. Again, some of the streams flowing into the Lovu river were too small to harbour crocodiles and yet the flies existed on the streams.

One very common source of food is afforded by several species of water birds, especially darters, which are accustomed to sit for hours on dead trees at the water's edge, with their wings widely outspread.

On the island in Lake Tanganyika, of which we have already spoken, the only living things were several species of birds, chiefly water-fowl, some snakes, mice, and a few crocodiles. This island lies at least two miles from the mainland, and has been uninhabited for, certainly, the past twenty years. Although *Gl. palpalis* was extremely abundant, and was found around the whole of the shore, the crocodiles were practically confined to one particular bay, so that all the fly did not have the opportunity of feeding on them. We attempted to feed captive flies on freshly-shot crocodiles, and while they made vigorous efforts to do so, they were unable to pierce even the thinnest portions of the skin of the particular crocodiles we were using.

About 33 per cent. of the flies we examined (61 out of 185), on this island, showed evidence of having fed on blood. In every case, with the exception of one, the blood cells, when recognisable, were oval and nucleated, but we were not able to determine, with any degree of certainty, whether they were from birds or reptiles. None of the birds or crocodiles we shot had blood parasites, *e.g.*, haemogregarines, and the changes produced in the contours of the cells and nuclei by the process of digestion rendered measurements useless. In the odd fly, the blood was human, and quite fresh, and had evidently come from one of the boys engaged in catching specimens.

41·7 per cent. of the *Gl. palpalis* were infected with intestinal parasites of the herpetamonad type. The discussion of these forms is reserved for a future paper.

##### V. HABITS OF *GLOSSINA MORSITANS*

So far as the time of day at which they are most active, their catholicity of taste in the matter of food, and their preference for dark colours are concerned, the habits of this species are much similar to those of *Gl. palpalis*.

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\* Koch, 1907. Deutsche med. Wochenschrift, Jahrgang 33, No. 46.



On several occasions we thought that they showed a more decided preference for khaki than for the dark skin of the native, but this may have been due to the fact that we walked ahead of the carriers and so attracted the fly first.

More or less seasonal variation is observed in the case of this fly, and in some localities apparently depends upon the winds, for during the South-west monsoon they cannot be found on many elevated spots where they exist at other times of the year.

When we reached the top of the plateau at the Lovu river, at the end of May, 1908, *Gl. morsitans* was present, but during August we were unable to get specimens, although there were plenty in the valley below. In October, when the winds had ceased, they could again be found at the edge of the plateau.

Some of the officials who have been many years in the country, deny that there is any great difference in the numbers of the fly in the wet and dry seasons. We were never long enough at any one place to make any observations on this point. One official said that he had noticed that the fly was absent in the part of his district where the soil was clayey, and that they could be found in surrounding areas where the soil was sandy. If this observation is correct, it might be explained by the larvae being unable to burrow into the stiffer soil in order to pupate. Another said that they could always be found in 'mopani' bush, i.e., forest composed of these trees, which are rather far apart and grow with straight trunks. The ground beneath them is covered with a scanty carpet of grass, devoid of undergrowth, as the soil is sandy and not very fertile.

*Gl. morsitans* requires much less shade than *Gl. palpalis*, and is not commonly found in such close connection with water as this species; indeed, they may be taken miles away from it.

Near Chongola's village, on the Luapula river, the country is one series of immense, bare, and absolutely level flats separated by very small patches of bush. *Gl. morsitans* abounds in this bush, and also in the small, bush-clad ant-hills which dot the plains. They fly from these ant-hills to attack the passing caravans, but are never seen in the absolutely bare flats, unless they have been carried there on the natives. We travelled through this country, in the dry season, for seven hours on one occasion, before reaching a water hole.

Ensor\* has noted that the two species have selective spheres and that they do not encroach on one another's territory, and while our observations agree, on the whole, with this, we have caught the two flies together under circumstances which would preclude the possibility of *Gl. morsitans* having followed us to the water.

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\* Ensor, 1908. Wellcome Lab. Reports, No. 3.



This was seen more particularly along the coast of Lake Tanganyika, in several of the small bays which were closed in by precipitous cliffs. We landed at these from a canoe, and caught both species together.

They are more resistant to changes of temperature than *Gl. palpalis*, and will flourish at much greater altitudes; in many places we have caught them well over four thousand feet above sea level. They are said to shun human habitations; this depends, apparently, to some extent on the character of the village, that is to say, whether it is clean and well-kept or whether the spaces between the huts are utilised to grow such crops as cassava, for in such we have caught *Gl. morsitans*.

For instance, they have come into the tent when pitched in a village, and have been caught in another while we were palpating the people.

This condition, however, is rather unusual in most parts of the country, so that it is rather rare to see the fly actually inside the villages.

It may be as well, in view of Koch's\* statement that *Gl. morsitans* in German East Africa will not attack men, to say that the Rhodesia and Nyasaland varieties do not hesitate to do so, and wherever they are met they become a veritable pest.

The pain felt when a tsetse fly bites is variable. It may be so acute as to feel like a red-hot needle, or, on the other hand, it may be quite painless. This depends altogether on the spot they select. As a rule, no after-effects are noticeable at the site of the bite. In those cases where the fly experienced any difficulty in getting through cloth, it would very frequently cause the wings to vibrate very quickly, with a sharp buzzing sound, as if it were trying to bore through the obstruction. This buzzing sound is most distinctive, and often is the first thing which draws the attention to the presence of the flies when they are very scanty and difficult to catch.

After making all allowance for lack of sufficient observation, there can be little doubt that *Gl. morsitans* is increasing in numbers in these countries. This statement is made by all the Europeans who have been in the territory for any number of years, and while many of them have only taken an interest in the question since Sleeping Sickness has become so prominent a subject, and may, therefore, be jumping to the conclusion, definite evidence exists in some cases.

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\*Koch, *loc. cit.*

One settler in Nyasaland, for instance, who kept cattle, had to abandon one locality owing to its invasion by fly, and is now finding that his second estate, which was free of flies when he took it, and on which he has been settled for several years, is very quickly being rendered useless to him in the same way.

There are large herds of cattle, belonging to Europeans, at Fort Johnston, at the south end of Lake Nyasa, and numbers of these used to be driven with impunity to Zomba some years ago. Since that time, however, the fly has spread to such an extent that it is no longer possible to do this.

In one district in N. E. Rhodesia, actual fly maps were made by a competent observer, and these show that the same thing is happening there.

This increase cannot be ascribed altogether to a corresponding increase in the amount of game, for the fly is found in many districts where this is very small, and in other cases the reverse holds good.

The Native Commissioner of the Serenje district of N. E. Rhodesia told us that there is practically no game in the area close to Chitambo's village, yet the fly are very plentiful there.

Along a road, in the Congo Free State, running parallel with the Luapula river, *Gl. morsitans* was present in large numbers, and yet there were no traces of game in the neighbourhood.

Near Abercorn, in N. E. Rhodesia, there are large herds of eland, hartebeest and roan antelopes, while tsetse flies are absolutely unknown. Other examples might be given.

It has been recently suggested that the occurrence of the flies may be cyclical, and, if so, it may account to a great degree for these facts. In Nyasaland, on the South Rukuru river, where tsetse flies are said to have been abundant twenty years ago, there are none at the present time, while they are returning to the Chobi river and Wankies, in Southern Rhodesia, from which they have been absent for some years. There is a native rumour that they are also returning to a portion of the Transvaal.

As in the case of *Gl. palpalis*, the food of *Gl. morsitans* will depend on the local fauna, and it does not follow, as has often been said, that wherever buffalo are found there will also be this fly.

On one occasion, on the west shore of Lake Bangweulu, we covered a piece of country in which a herd of from fifteen to twenty buffalo habitually lived, without meeting with the fly anywhere, even close to the herd.

If buffalo are found in country where *Gl. morsitans* exists, it is altogether natural that the flies should be more abundant in the neighbourhood of the animals, as they do not travel quickly, are local in their habits, remain practically always under cover, and afford an abundant supply of food. Farther than this, we do not think there is any closer connection between this particular or any other species

of game. In the country itself, opinion is divided on the question of the inter-relation of tsetse flies and game. Some men state most positively that there is no close connection at all between the two. Others say that buffalo and the fly are always seen together; others that elephant are the selected animals; others that kudu; and so on. These conflicting statements can be explained in part by incorrect observation and in part by prejudice.

## VI. GLAND PALPATION AND PUNCTURE

During the period February 24th to June 15th, 1908, we palpated 17,923 natives, of whom 3,056 had palpable glands (post cervical), and with our previous figures the totals are 26,928 and 4,934, so that about a fifth of the people (17.05 per cent.) present the condition. These glands are classified\* as follows:—

' + '	29, or 0.58 per cent.
' + - '	115, or 2.33 per cent.
' + - - '	4,791, or 97.24 per cent.

The results of puncture were:—

Class	No. found	No. punctured	No. infected	Percentage success
' + ' ...	29† ...	29 ...	21 ...	72.4
' + - ' ...	115 ...	108 ...	1 ...	0.9
' + - - ' ...	4791 ...	608 ...	0 ...	0.0

Amongst the ' + ' class we have included two cases in which symptoms of tuberculosis were present and in which the gland juice was purulent, so that when these are excluded the percentage of successful punctures becomes 77.7. It should be noted that all the

\* The classification to which we have adhered is that used by Dutton and Todd, viz.:

' + ' glands, (a) one gland, estimated size  $1.5 \times 0.75$  cm.

(b) several (3 or more) smaller glands, the largest measuring perhaps  $1 \times 0.75$  cm.

' + - ' glands, showing less enlargement than ' + ' but more than ' + - - '

' + - - ' glands, (a) only one or two glands measuring  $0.5 \times 0.25$  cm.

(b) many tiny, usually hard and shot-like glands, which were only just palpable.

† In two of these cases, negative on gland puncture, and in which there was no apparent cause for the enlargement, the blood and cerebro-spinal fluid were centrifuged and examined, but with negative results.



natives were seen only once, and it is possible, therefore, that had we been able to examine them more frequently a still greater percentage of them might have been found to be infected.

A word may be said here with reference to the classification. In all our ' + ' cases the enlargement was relatively enormous, so much so that in several of them the glands could easily be recognised, on casual inspection, as irregular swellings at the sides of the neck. We had the opportunity of seeing the great majority of the other cases found in the country, and in these the enlargement was also extremely well marked, so that there could not be any hesitation in assigning them to this particular class. All the glands were freely moveable, separate from one another, and of the peculiar, and almost diagnostic, consistency which Hodges\* has aptly compared to that of a ripe damson.

In the one positive ' + - ' case we were led to suspect the presence of the disease by this feature alone, before puncture had revealed the trypanosomes in the gland juice.

The ' + - - ' class included glands which are usually few in number, and which are very small and hard—like BB shot. They are most commonly found at the apices of the posterior cervical triangles, and as we have already pointed out, have no specific import, so that a clear distinction may be drawn between them, as being merely ' palpable ' as opposed to ' enlarged.'

The intermediate, or ' + - ' class, we have found to be of little use, and our results point to the conclusion that this group is perhaps superfluous. In any such arbitrary classification, however, so much depends upon the interpretation of the individual that it is as well that a somewhat smaller measurement should be adopted than the one which would indicate with any degree of certainty the presence of trypanosomes. This means but little extra work, and increases the efficiency of the method. Our experience bears out that of Dutton and Todd,† and of Martin and Leboeuf,‡ that the larger the glands the more likely is one to find trypanosomes, that is in early cases of the disease.

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\* Hodges, 1907, Colonial reports, Uganda, No. 4662.

† Dutton and Todd, 1906, Liverpool School of Tropical Medicine, Memoir XXI.

‡ Martin and Leboeuf, 1908, Bulletin Soc. Path. exotique T., No. 8.



The glandular enlargement may not be general, for in our cases it was the exception to find that the axillary and epitrochlear glands were enlarged concurrently with those in the neck. We have not considered the inguinal glands, as in practically every native there was a noticeable degree of enlargement in these groups.

Some idea of the length of time required for the enlargement to attain a marked degree is afforded by the fact that some of our cases, found on Lake Tanganyika, with decidedly ' + ' glands, had been seen by Spillane some six months before, and had not been punctured by him on account of the small size at that time. We were informed by one of the medical officers engaged in Sleeping Sickness work in Rhodesia, that he had seen one case in which the enlargement had occurred within three months.

In an endeavour to account for the slight degree of enlargement which is so common, we recorded the approximate age\* and the sex of many of the natives (18,512), and the relation of these factors may be seen in the following table:—

Age	MALES			FEMALES		
	No. palpated	No. glands	Per cent.	No. palpated	No. glands	Per cent.
0—10	2589	1128	43·5	2944	752	25·5
11—20	1509	434	28·7	1104	175	15·8
21—30	2331	439	14·5	3444	229	6·6
31—40	2066	184	8·9	2090	85	4·0
Over 40	269	7	2·8	186	15	8·0

The gradual decrease in the incidence of palpable glands with advancing age is very marked, and corresponds to a large degree with the general diminution in the lymphoid tissue of the body which is known to occur at this period. It is, however, not clear why there should be such a noticeable difference in the occurrence of enlarged glands in the two sexes. The enlargement is encountered in all parts of the country, and is not related in any way to the distribution of

\* We have assumed that the age of puberty is about 12, and while we endeavoured to estimate the ages of the natives as correctly as we could, we cannot vouch for the absolute correctness of them. We believe that by dividing them into decades we have succeeded in approaching some degree of accuracy.

biting flies, tsetse more particularly. To a great degree it may be attributed to general dirtiness, which is always more marked amongst the children.

At one village, near the Luangwa river, where the natives were particularly dirty, we were told that they did not wash, as they were afraid of catching cold.

Conjunctivitis and purulent discharges from the nostrils are extremely common in babes. In some districts, skin diseases, such as 'mperi,' or itch, are frequent, and in others, e.g., amongst the Ankonde at the north end of Lake Nyasa, filariasis may be responsible to some extent. There, nearly every man has some degree of lymph scrotum, and filaria may be found in the juice of the lymphatic glands. It has also seemed to us that there are tribal differences, for in those which are comparatively cleanly, e.g., the Awemba, the smaller incidence is quite noticeable. This supports the supposition of the enlargement being due largely to general filthiness.

Since Dutton and Todd\* laid such emphasis on gland palpation and puncture, particularly in the early stages of the disease, considerable discussion has arisen not only as to its value as a method of diagnosis, but also to the efficiency of the measures of prophylaxis based on its application. Their original conclusions were:—

1. 'Gland palpation is by far the most efficient method of demonstrating the presence of trypanosomes in cases of trypanosomiasis.'

2. 'As a rule, enlarged cervical glands, without obvious cause, do not occur in districts from which trypanosomiasis is absent,' with the corollary that 'every negro with enlarged glands must be considered, until the contrary is shown, to be a case of trypanosomiasis.'

3. 'Early cases of trypanosomiasis have enlarged glands, and will, therefore, be detected by gland palpation.'

As in the countries with which we are dealing the detection of the early cases is the end in view, we shall restrict our attention as far as possible to these.

1. 'Gland palpation is far the most efficient method . . . . .'

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\* Dutton and Todd, 1906, Liverpool School of Tropical Medicine, Memoir No. XXI.

Dutton's and Todd's figures are, for early cases :—

Gland puncture	...	...	...	...	98.5 % successes
Blood (partly ordinary preparations, partly centrifuged)...	31.4 %	„			
Cerebro-spinal fluid	...	...	...	...	13.0 % „

The results obtained by Greig and Gray\* approach very closely those just given, but as cases in all stages of the disease are included, they are not very valuable for our purpose :—

Gland puncture	...	97.0 %
Blood	...	52 %
Cerebro-spinal fluid	...	84.9 %

Koch's† earlier results gave a percentage of 97.4 successful punctures, and he states 'dass dieses Symptom (glandular enlargement) bei den Trypanosomiasis-Kranken ein ganz constantes ist, das nicht wie das Auftreten der Trypanosomen im peripheren Blutstrom starken Schankungen unterworfen ist.' When he found that the trypanosomes were banished from the glands by the use of Atoxyl he was compelled to revert to the examination of the blood in order to control the treatment, and then found that by his improved method, he was able to detect the parasites in 40 per cent. of the cases by a single examination, and in practically all of them after the fifth. He goes on to say that where the examination of large bodies of natives is in question his method would occupy too much time, except in the case where the treatment is being controlled, and adds that for quick diagnosis gland puncture must remain the most practical (zweckmässigste).

\* Martin and Leboeuf‡ repeated Dutton's and Todd's work in the French Congo, and their results differ to some extent for cases 'en bon etat' :—

Centrifuged blood	...	100.00 % successes (6 cases)
Gland juice (complete)	...	90.56 % „
Cerebro-spinal fluid	...	28.57 % „

Their technique in centrifuging the blood was most thorough, involving three separate centrifugations, occupying in all between

\* Greig and Gray, 1905, Roy. Soc. Sleeping Sickness Reports, No. VI.

† Koch, 1907, Deutsche med. Wochenschrift, Jahrgang 33, No. 2.

‡ Martin and Leboeuf, 1908, Bull. Soc. path. exot. T. I, No. 2.



threequarters of an hour and an hour. They return to the question in a second paper,\* with practically identical results:—

Centrifuged blood	...	100.00	% successes (12 cases)
Gland juice	...	91.46	% „
Cerebro-spinal fluid	...	26.31	% „
Finger blood	...	36.66	% „

There is rather a remarkable falling off in the blood examinations in their 'suspected' cases, 85.71 per cent., as compared with 90.90 per cent. for gland puncture. They conclude, however, that their results are practically the same as those of Dutton and Todd, and that while gland puncture is the best method to employ in travelling, the examination of the blood must be practised in treatment.

Our results from the direct examination of the peripheral blood (fairly thick fresh preparations, using  $\frac{3}{4}$ " square cover glasses, Zeiss D objective, and No. 4 eyepiece) agree with those of Thiroux, Wurtz and Teppaz,† that is, in ten cases trypanosomes were not seen. Only a single examination was made in each case, however, and the number is too small for any exact deductions.

In considering the efficiency of gland palpation, it is essential to know with what degree of certainty one may depend upon finding the trypanosomes in cases of the disease, at any or every examination. Gray and Tulloch‡ say that 'trypanosomes are constantly present in the lymphatic glands of early . . . cases . . . and can be found on any day . . . ' Koch also, as we have said, points out much the same thing.

If we were dealing with known cases of the disease, it might be concluded that centrifugation of the blood is the most efficient means of diagnosis, though it must be said that the number of cases on which this conclusion would be based is rather small.

In any one of the methods there is naturally an appreciable margin for error, and not only so, but the thoroughness with which they are practised will affect the result to a great degree. When we come to deal with a country in which the disease is not endemic and in which the examination of large numbers of natives is involved, and

\* Martin and Leboeuf, 1908, Bull. Soc. path. exot., T. I, No. 8.

† Thiroux, Wurtz and Teppaz, 1908, Bull. Soc. path. exot., T. I, No. 5.

‡ Gray and Tulloch, 1907, Roy. Soc. Sleeping Sickness Reports, No. VIII.



where, moreover, the men engaged in the work are not furnished with an elaborate equipment, there can be little doubt that the method of gland palpation and puncture is the most serviceable method and the one which must be adopted as a routine measure.

2. 'As a rule, enlarged cervical glands, without obvious cause, do not occur in districts from which trypanosomiasis is absent.'

Low was the first to dissent from this statement, and he pointed out that enlargement of the glands was common amongst natives, unassociated in any way with Sleeping Sickness. The truth of the above dictum depends almost altogether on the meaning which is attached to the word 'enlarged.' If this is taken to mean any gland, however small, which can be distinctly palpated, the observations which have been made on the question, enable us to say at once that there is very little truth in it. If we are to take it that by 'enlarged' we are to understand glands measuring approximately  $1.5 \times 0.75$  cm., the case is different. The figures given by Dutton and Todd lend some colour to this supposition, for of 157 ' + - ' and ' + - - ' glands punctured by them, both in infected and uninfected regions, only two were found to be infected. A distinction must be drawn between infected and non-infected regions, for in the former, as Dutton and Todd,\* Neave† and others point out, glandular enlargement is much more common than in the latter, and, in addition, glands of the larger class are much more abundant. Neave says 'This would tend to show that trypanosomiasis is accountable for enlargement of glands of any size.'

On the whole, our results in Rhodesia, a supposedly non-infected region, bear out those of Dutton and Todd. As we have already pointed out, glandular enlargement in the wide acceptance of the term has no particular significance, but in the case of glands of the ' + ' class, in four out of five examinations the natives showing them were infected with trypanosomes.

3. 'Early cases of trypanosomiasis have enlarged glands, and will therefore be detected by gland palpation.'

That the first part of this statement is correct is recognised by all workers. Greig and Gray‡ state that 'the disease is at first a specific

\* Dutton and Todd, 1906, Memoir XXI, Liverpool School Trop. Med.

† Neave, 1908, Report of Katanga Medical Commission.

‡ Greig and Gray, 1905, Royal Soc. Sleeping Sickness Reports, No. VI.

polyadenitis.' While this is broadly true, glandular enlargement is not an absolutely constant sign in the early stages of the disease, for cases have been diagnosed in which the glands had not become palpable. About 8 per cent. of known cases of trypanosomiasis will not be detected by gland palpation.

The great divergence of opinion that exists, is, in regard to the application of gland palpation to methods of prophylaxis; the chief objections being that it fails to detect an appreciable number of the cases, that it entails hardship on those natives unfortunate enough to have big glands, that the natives will evade its application, and that it is an expensive scheme to carry out. The second and third of these items need not be considered, as the objections cannot properly be against the method; since, if they occur, they are attributable to injudicious handling of the natives by the officials concerned in the work. The objection that all the cases will not be found by the method is valid. In order to stamp out the disease in a district, the ideal procedure would be to stop absolutely native movements, and to prevent the infection of fresh natives in the area, by the isolation of every case of the disease and the removal of the remaining population from contact with tsetse flies, *Glossina palpalis* more particularly. Since it is manifestly impossible to carry any such scheme entirely into effect, we have to revert to measures which will tend to check the spread of the disease, even though these be imperfect. Dutton and Todd do not claim that their procedure will entirely prevent Sleeping Sickness from spreading; all they say, is, that it will prevent the disease from infecting new districts with the rapidity noticed in the past, and in the meantime improved methods both of prophylaxis and treatment may be brought to light. So far as treatment is concerned, we are in much the same position as we were four or five years ago, that is to say the results are not at all promising, so that we are still compelled to fall back on imperfect methods of checking the advance of the disease. If natives are to be allowed to pass from infected to non-infected regions, the fact remains that no better method of detecting the disease in the early stages has yet been put forward than the application of gland palpation. Of course to complete the method, gland puncture must be employed as well, and this has always been stated to be an integral part of the procedure. It is surely better to stop the nine out of ten cases, which the method

will detect, from travelling, than to allow the whole ten to go, just because the odd one will be missed at the time. If this tenth man were registered on leaving his home, and required to present himself for examination at stated intervals, the disease would probably be diagnosed before sufficient time had elapsed for him to become a source of danger in his new surroundings.

This brings up the question of the registration of the native population. Wherever this is feasible, it is desirable that it should be done, as in no other way can the native be so effectually controlled and traced. It is an important preliminary step to the adoption of a pass system.

In North-Eastern Rhodesia, where the conditions are perhaps more favourable than in those countries in which *Gl. palpalis* is found everywhere, the scheme of fighting Sleeping Sickness, based on the application of gland palpation and puncture, is in full working order, and is apparently being accompanied by a considerable amount of success.

The question of the expense depends altogether on whether the eventual results will justify its incurrence. We believe that to obtain the fullest benefit, special medical officers, who have some experience in the work, will have to be appointed, and of course this is rather costly.

In tropical Africa, the development of the country is dependent upon a plentiful supply of native labour, and in some parts, at least, the greater part of the revenue is derived from the imposition of hut taxes; so that it is an important matter that as great a percentage as possible of the natives should be in a position to work. It is certain that if the disease is allowed to go on spreading, the development of many of the African colonies will come to a standstill, so that the cost of any measures designed to check the advance of Sleeping Sickness will be cheap in the end. This is a matter which must be settled in each country, as it will depend upon the area to be guarded, upon the density of the population, and upon the amount of traffic which is allowed to go on.

We must confess that from what we have seen in Rhodesia we are in favour of gland palpation. We believe that, combined with gland puncture, it is a most useful measure, and one which will render real service in preventing any rapid extension of the disease



to 'clean' areas. Within the infected areas, it is the most practical method of isolating infected natives. The conditions present in the particular country concerned may possibly affect the results obtained, but if it is seriously and efficiently applied, we consider that the results will more than justify the cost.

#### VII. OCCURRENCE OF CASES

The Principal Medical Officer, Dr. Spillane, in a tour made during October and November, 1907, found twenty-six cases of the disease in North-Eastern Rhodesia, two in villages on the Mansa river (a tributary of the Luapula), eleven in the vicinity of Lake Mweru, and thirteen on Lake Tanganyika. About the same time, we found three cases in villages near the Luapula, and during our later trip we diagnosed eighteen more, two on Lake Mweru and sixteen on Tanganyika. With a few additional cases, which have since been found by the various medical officers, there are between fifty and sixty known ones in the country.

The one case found in Nyasaland has an interesting history. It is that of a boy who had accompanied his master from that country down the Congo river to its mouth. From there he had gone to Cape Town, and finally returned to his home by way of the Zambesi. This illustrates the extent to which some of these natives will travel in the employ of Europeans.

Only four of our cases were in women. The remaining seventeen were in males varying in age from ten to forty years. The great majority of them were adults. With the exception of two, in whom clinical symptoms were present, all were apparently quite healthy, and the only sign observable was enlargement of the lymphatic glands in the neck.\*

Some of them said that they had had occasional attacks of fever and headache; others that they had not been ill at all. Headache is, however, such a common complaint amongst natives, due usually to constipation, that no attention need be paid to it, unless it is exceptionally severe.

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\* These cases were seen only once, so that we are unable to say whether there were any slight disturbances of the body temperature or the pulse which may be present in otherwise apparently normal cases of the disease. So far as we could determine at the time, the natives we found to be infected were quite free from any symptoms.



It was an everyday occurrence to have carriers come up complaining of 'mutu,' or headache, and this could almost always be relieved by a dose of some purgative.

No loss of strength, emaciation, skin eruptions, tremors or mental disturbances were noticeable. In the two clinical cases, tremors, emaciation, and inability to walk were the principal symptoms present. We never saw a case sufficiently advanced to show mental symptoms or coma.

Insanity is said to be a common early symptom of the disease, but in two cases which we saw, and on which we performed lumbar puncture, no parasites were found in the cerebro-spinal fluid.

### VIII. MODE OF INTRODUCTION

We have stated in our previous report that some of the cases on the Luapula river have apparently brought the disease into Rhodesia by way of the Katanga. The foci on Lakes Mweru and Tanganyika are due to direct extension from the immediately contiguous infected areas in the Congo Free State. On the Mweru side, the disease has been gradually spreading up-stream from the Congo, along the Lualaba, until it reached Lake Mweru probably four or five years ago. In the Congo Free State, on Lake Tanganyika, imported cases existed at Moliro, just over the international boundary, in 1901; at Baudoinville in 1902; and at Vua, between these two places, about the same time. Within the last two or three years such a large percentage of the native population, along this portion of the lake, has died from Sleeping Sickness that the White Fathers have been compelled to abandon their missions.

On both lakes, the tribal and political boundaries do not coincide, and on both, the paramount chiefs (Mpweto and Moliro) live in Belgian territory. There has always been constant communication between the people on either side of the line, and to this the introduction of the disease into Rhodesia has been due. In eliciting the past history of our cases it was extremely common to find that a native had been born in a village on the British side, had then been taken as a child into the Congo, had afterwards returned to our territory, and finally settled down in a fourth village. The same may be said of the villagers on the eastern side of Lake Tanganyika, except that here the movement was into German East Africa. On

both Lake Mweru and Tanganyika, the most heavily infected villages were those directly contiguous to the Congo Free State. The further extension of the disease has been aided by the free communication, chiefly in canoes, which has been going on from village to village.

Although there has been free communication with German East Africa, it is rather improbable that many of the cases were contracted there, as the disease was introduced into that country (i.e., the eastern shores of Tanganyika) from the Congo Free State\* about the same time that it was, in all probability, introduced into Rhodesia, that is to say, not later than 1905. Comparatively few cases have been reported from the Bismarckburg section of German East Africa, which is the portion bounding North-Eastern Rhodesia.

Since its introduction into British territory, it has almost assumed endemic proportions on Lake Tanganyika. In November, 1907, twelve cases were found; in June, 1908, sixteen additional ones; and others since then; so that there is (or was, as they have since been moved) hardly a village on the lake shore in which one or more infected natives has not lived.

We have seen enough on this lake to make us realise very fully the great importance of international co-operation in dealing with Sleeping Sickness. However good a system of fighting the disease may exist in any one country, its efforts are sure to be retarded if the neighbouring territories remain apathetic. When the authorities in Rhodesia commenced to isolate the infected, some of them, and in one or two cases whole villages, immediately decamped over the border, where they not only constituted a source of danger to their new country, but remained one to that which they had left, for the probability existed that they would find their way back to their old villages as soon as they thought the vigilance of the authorities had relaxed.

#### IX. PROPHYLAXIS

From the reports published in various parts of Africa it is quite evident that it is useless to attempt to advance a system of prophylaxis which will apply equally well to every country. Certain well-defined methods of procedure exist, but so many factors have to be

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\* Colonial reports, German East Africa, for the year 1905-6. No cases of Sleeping Sickness had been found in the Bismarckburg section at this date.

considered that each country has to make a choice of those means which are best suited to the local conditions. For instance, in a country like the Congo Free State, where *Gl. palpalis* is omnipresent, it would be impossible to move the population away from it, so that such measures as clearing would have to be adopted. In Rhodesia, on the other hand, it has been possible to adopt rather drastic measures, partly owing to the physical configuration of the country, partly on account of the restricted distribution of *Gl. palpalis*, partly on account of the scantiness of the population, and partly on account of the lack of any very great commercial movement amongst the natives.

At one time native transport was an important item, as all goods passing into the Katanga and eastern portions of the Congo Free State went through the country, but with the advent of the railway in the South, and the adoption of the Congo river as the transport route to the East of the Free State, these movements were interrupted. Except for the inter-tribal communication along the border, there is no inducement for the Rhodesian natives to go into the Congo. A certain number did go to the Katanga mines for work, but this was never very popular and never affected any great number. The more natural outlet for the labour has been to the South.

Before we left England in May, 1907, we suggested, on general grounds that natives of North-Eastern Rhodesia should not be allowed to seek work in the Katanga, and that the Luapula river should be closed for traffic. The advisability of this measure became still more apparent very shortly after we had entered the country, particularly as several cases of human trypanosomiasis had been found amongst natives who had been in that portion of the Congo Free State, and who had apparently contracted the disease while there. Consequently in November of the same year we insisted more strongly on the importance of the step, and at the same time suggested that the canoes along the Luapula river should be confiscated, that the Congo Free State authorities should be requested to co-operate in this work, that all cases of the disease should be segregated, and in order that this might be done that special medical officers should be appointed for Sleeping Sickness work, that villages should be removed from the vicinity of *Gl. palpalis*, and where this was impossible that clearings should be made. In December, and again



in our first published report, we reiterated these suggestions with a few additional ones of minor importance. The regulations which were finally adopted were much along these lines, and are, briefly:—

1. The Congo Free State and German East Africa are regarded as infected countries, and communication with them has been stopped.

2. All villages, except in two or three instances, have been moved away from contact with *Gl. palpalis*.

3. Certain districts of the country are regarded as infected, and communication between them and the other portions of the territory prohibited. These infected areas are three in number, one including the country around Lake Tanganyika, the second that around Lake Mweru, and the third that along the Luapula river.

4. Three special medical officers have been appointed, one for each of the infected regions, to travel constantly and search for cases of the disease.

5. All cases of human trypanosomiasis are segregated in special camps, in fly-free areas, for treatment.

6. All canoes have been confiscated, and fishing on 'fly' (i.e., *Gl. palpalis*) waters forbidden.

7. Clearings are made at necessary places, e.g., around the few villages which have been allowed to remain on the shores of Lake Mweru, and at several ferries.

The vigorous enforcement of these regulations seems to be meeting with success, for from November, 1908, to January, 1909, no further cases of the disease were found. The efficiency of the scheme depends upon the application of gland palpation and puncture, and, so far as one can see, it is working satisfactorily, but some time must necessarily elapse before a definite opinion can be given as to what the ultimate result will be. Apart from the transmission of the disease by coitus,\* and the question of whether any other tsetse fly than *Gl. palpalis* is a natural transmitter of *T. gambiense*, this will largely depend upon the personnel of the staff, and in this connection we cannot do better than quote the remarks of Neave†:—

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\* Koch, 1907, Deutsche med. Wochenschrift, Jahrgang XXXIII, No. 46.

† Neave, 1908, Report of Katanga Medical Commission.



'The personnel will have to have their hearts in the work, and  
'spare no pains or energy. Those who work just sufficiently to  
'conform to the letter of their agreements with their employers, with  
'the mere object of getting their pay and returning home with the  
'least expenditure of exertion, will be worse than useless, as the fact  
'of their being on the spot will lull the anxiety of the world, while  
'the disease will progress onwards round them. The work has a  
'tendency to be disheartening, and it is only those properly qualified  
'men who will doggedly spend all their time in the cause that are  
'likely to succeed, and then only with a knowledge of the native,  
'together with that peculiar tact and firmness necessary in the treat-  
'ment of him.'

One or two specific dangers may be pointed out, and these are, that as the natives on the Congo side of the borders have not been moved, nor deprived of their canoes, there may be some temptation for the British natives to go back to the rivers. The co-operation of the Belgian authorities should be requested in order to more fully safeguard these, as yet, practically uninfected regions. There may also be a tendency for some of the natives to return stealthily to the rivers, the Luapula more particularly, in order to fish with weirs, and to plant crops; this can only be safeguarded by periodical inspections. Unfortunately, there is also a necessity to guard against the short-sighted policy displayed by some Europeans in attempting to make the natives evade the regulations when it suits their convenience, in spite of the penalties which are attached to infringements of the law.

There is not much danger of the natives in the 'infected' areas doing any more than simply crossing the boundary for a very short distance, for it so happens that the division between the infected and clean portions is practically a tribal one, and this is very advantageous.

In Nyasaland it is altogether probable that other cases similar to the one which has been diagnosed are present. Many of the natives, particularly of the Mombera and West Nyasa districts, have worked in the Katanga; when we were at Madona we saw a number of them passing through on their way back home. Not only this, but as most of the skilled labour and personal servants in the East Central portions of Africa are natives of this Protectorate and travel much more freely in search of work than the raw native, as in the case recorded above, many of them have been in dangerous localities.

These cases can only be found by a systematic search by properly qualified medical men.

At the same time, medical men can do other most useful work in mapping out the distribution of various biting insects, and conducting preliminary enquiries into the incidence of cattle and other diseases. However, it should be distinctly understood that the work in connection with Sleeping Sickness is sufficiently arduous to require undivided attention, and the tendency which occasionally is seen to add this work to the duties of the ordinary district medical officers can only result in the work being indifferently executed.

This also applies to the clean areas of N.E. Rhodesia, for it must not be forgotten that the first case of human trypanosomiasis diagnosed there was at Chinsali, on the Tanganyika-Nyasa plateau, where no *Gl. palpalis* exist.

The natives should be encouraged to apply for passes when they are about to travel. Provided no unnecessary difficulties are placed in the way of these being obtained, the natives will, in time, apply for them as a matter of course. We found that whenever we had occasion to send a messenger, he would always come up and ask for a note before leaving.

The eventual fate of both Rhodesia and Nyasaland, to say nothing of other territories which bound them, depends on what species of tsetse flies can convey the disease in nature. *Gl. palpalis* has only a comparatively small distribution, so far as we know, but that of *Gl. morsitans* is extremely wide, so that if this species can transmit *T. gambiense* the greatest danger exists.

It is admitted that mechanical transmission is possible, but whether it plays a very extensive rôle in nature, in determining the spread of the disease, is questioned. In any case, the odds against it occurring are enormous, for even in laboratory experiments with *Gl. palpalis*, the number of flies which has been required for a successful result is extremely large, and the conditions under which these experiments have been carried out in the laboratory would occur but very seldom in nature. Whether, however, the position that the possibility of it happening in nature can be ignored, is rather questionable. We believe that mechanical transmission is responsible for the spread of certain cattle trypanosomes, and some support for the supposition that it may take place in the human disease is afforded

by the observations of Martin, Leboeuf and Roubaud on the occurrence of hut-infections, which they are inclined to attribute to the agency of such domestic mosquitoes as *Stegomyia* and *Mansonia*. That *Stegomyia* will transmit *T. gambiense* we know.\*

With *Gl. morsitans* the probability of mechanical transmission happening is perhaps slight, but the danger will be proportionate to the number of cases which are present. This fly is not nearly so local in its habits as *Gl. palpalis*, and consequently is not afforded the same opportunities of acting as carrier. It is very surprising to notice, even in the case of *Gl. palpalis*, the slow rate at which the disease spreads under the most favourable circumstances. In two or three villages on Lake Tanganyika where this fly, as well as *Gl. morsitans*, was caught in the gardens, and into which the disease, in all probability, was introduced four or five years ago, only nine or ten cases of the disease were found, and if this is so, it is quite conceivable that the rate of spread, in the event of *Gl. morsitans* being an *active* transmitter, as opposed to a *mechanical* one, would be much slower.

*Gl. palpalis* is restricted to the immediate vicinity of water, so that it has constant opportunities of feeding on the natives when they come to wash, fish, and draw water. *Gl. morsitans* is not restricted to these localities, but is usually found in the bush, and, in general, avoids the villages and the gardens which surround them. Consequently the natives are not exposed to the bites of this fly in the same way as they are to those of *Gl. palpalis*.

On the other hand, it cannot be regarded as settled that *Gl. morsitans* transmits merely mechanically. In some parts of Africa, *Gl. palpalis* transmits various cattle trypanosomes which in other localities are carried by *Gl. morsitans*, and other examples of the same nature might be cited. If we institute analogies with other protozoa, we see that malaria, for instance, is not transmitted merely by one particular species of mosquitoes, but that even many different genera are implicated. The same thing is seen in the case of redwater and ticks. Recently Kleinet† has made experiments which suggest that a cycle in the life of trypanosomes does occur in tsetse

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\* Fülleborn and Mayer, 1907, Archiv. f. Schiffs- und Tropenhygiene, Bd. XI, Heft XVI.

† Kleine, 1909, Deutsche med. Wochenschrift, March 18.

The confirmation of this work by Bruce, who used in his experiments *Gl. palpalis* and *T. dimorphon* and *T. gambiense*, is an additional argument for using *Gl. morsitans* in a similar set of experiments.



flies, and it should be noted that he was working with *Gl. palpalis* and *T. brucei*, which is not ordinarily transmitted by this species. He thinks that, in nature, *Gl. fusca*, by which *T. gambiense* has been transmitted experimentally, spreads human trypanosomiasis.

The principal argument that *Gl. palpalis* is the natural carrier of Sleeping Sickness is that the geographical distribution of the two coincide to a great extent, and this is certainly a most striking fact. The waterways have always been used as highways in preference to land routes, and also as sources of food, so that the spread of the disease along them has been greatly facilitated by the abundant opportunities afforded the very local *Gl. palpalis* to feed on both infected and uninfected people. Even in those cases in which other species of tsetse flies have been found in association with *Gl. palpalis*, the possible share they may have had in the spread has been overshadowed by that of this particular variety. It is only within the last three or four years that the disease has spread far enough to come in contact with other species of tsetse flies, *Gl. morsitans* more particularly, in territory where *Gl. palpalis* does not exist. As we said above, we should naturally expect that transmission by this fly would go on very much more slowly than by *Gl. palpalis*. The question can only be settled by means of a patient and thorough investigation; it is one which should be carried out without delay. If it can be shown conclusively that *Gl. morsitans* will not transmit, except mechanically, the saving in expense to the various administrations concerned will more than balance many times the cost of the work, and if, on the other hand, it should be shown that *Gl. morsitans* is a *natural* carrier, there will yet be time to take more active steps to protect the threatened countries from invasion. Until the question is answered decisively, the possibility must never be forgotten, and must be acted upon to some extent.

In conclusion, we may refer to the conditions under which labour is allowed to leave both Rhodesia and Nyasaland. Quite recently arrangements have been made with the administration of Southern Rhodesia that no labourers from the British colonies to the north shall be accepted unless provided with Government passes. In order that the natives can obtain these passes, they must be examined medically, and in addition are examined a second time when they have reached their destination. No natives from a Sleeping Sickness



area are allowed to leave their districts. This scheme acts not only as a bar to the possible escape of cases of trypanosomiasis to the south, but will also control to a large degree the spread of other communicable diseases and prevent the employment of physically unfit labourers. The scheme is completed by the Southern Rhodesian Government requiring all labourers to be registered, and the employment of unregistered natives being made an offence.



# A FURTHER REPORT ON TRY- PANOSOMIASIS OF DOMESTIC STOCK IN NORTHERN RHODESIA (NORTH-EASTERN RHODESIA)

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## SUMMARY OF CONTENTS.

	PAGE
I. Introduction .....	312
II. Distribution of Stock.	
(a) North-Eastern Rhodesia .....	313
(b) Nyasaland .....	317
III. Occurrence of Biting Flies.	
(a) <i>Glossina palpalis</i> .....	319
(b) <i>Glossina morsitans</i> .....	319
1. Distribution .....	319
2. Association with 'Game' .....	323
3. Relation to Disease .....	328
(c) Other biting flies .....	331
1. <i>Tabanus</i> and Disease .....	331
2. <i>Stomoxys</i> and Disease .....	331
IV. The Disease in Stock .....	334
V. Prophylactic measures recommended .....	337
VI. The Trypanosomes encountered.	
(a) In Naturally-infected Ruminants .....	340
1. Scotsdale ( <i>T. nanum</i> ) .....	340
2. Kambole .....	348
(a) 'Balungu' ( <i>T. congolense</i> ) .....	349
(b) 'Ninamwenda' (sp. ?) .....	354
(b) In Naturally-infected Dogs .....	358
1. 'Chunga' (sp. ?) .....	358
2. 'Wallace' ( <i>T. congolense</i> ) .....	364
3. 'Jock' ( <i>T. congolense</i> ) .....	366
(c) In Naturally-infected Pig (sp. ?) .....	367
VII. Examinations of 'Game' .....	370
Occurrence of Trypanosomes .....	371
VIII. Supplementary Note to our 'Report on Trypanosomiasis of Domestic Stock in North-Western Rhodesia' .....	373

## I. INTRODUCTION

The members of this expedition reached Broken Hill in June, 1907: one of us (A. K.) shortly afterwards proceeding to Fort Jameson and thence to Madona on the river Luapula. The other (R. E. M.) remained at Broken Hill until October, investigating the trypanosome disease in cattle, and then proceeded along the river Kafue and through the corner of the Congo State to Madona, where the first meeting was effected in December. In February a joint tour was made of Lake Bangweolo; A. K. then travelling North via Luena, Kalungwisi and Chienji, and West to Lake Tanganyika and Abercorn. R. E. M. proceeded southwards along the outlet of the Luapula river from Bangweolo and thence North via Mpika, Chinsali and Kasama to effect a second meeting at Abercorn in June, 1908. In August and September the latter made a tour of the Stevenson Road, visiting Kawimbi, Ikomba, Mwenzo, Fife and Chinsali, for a second time, returning to Kambole, where an experimental camp had been built, via Abercorn.

Orders to return to England were received in November, and on the 15th we left Kambole. A. K. proceeded to the coast via Fife, Karonga, Blantyre and down the Shire River; R. E. M. attended the Pan-African Veterinary Conference at Pretoria as delegate of the School, and travelling via Kasama, Mpika, Serenji and Broken Hill reached that place on January 9th, 1909.

Our unavoidable recall to England before the experiments on transmission were fully established is to be regretted, as it was the first occasion since leaving the railway that we were together and were able to remain in one place for more than a few consecutive days, and to attempt anything like continuous experimentation. As a result our observations are necessarily limited and unfinished, and the paramount questions as to whether flies other than tsetse are to be held culpable for the transmission of the trypanosomes we encountered; whether 'game' is the main natural reservoir for these trypanosomes, and whether tsetse are unable to exist without 'game' still await final solution.

We have already reported our observations at Broken Hill: our results on similar conditions in North-Eastern Rhodesia are recorded here together with a summary of our notes on the distribution of



*Glossina morsitans* and its relation to wild animals. Passing through a country, as we were, it is difficult to place a correct value upon isolated observations; but since our results tend to coincide in many important particulars with those of certain observant residents, and are somewhat opposed to the popularly held beliefs which have been imported from South of the Zambesi, it is deemed advisable to set them forth here for whatever worth they may possess.

The strains of trypanosomes we obtained were carried to Pretoria en route for England, but it was found there that the steamship line by which we were returning refused to permit them to be taken on board. We consider ourselves fortunate that it was to Pretoria they had been brought, for Dr. Theiler has most kindly undertaken to look after them until further arrangements are made.

## II. DISTRIBUTION OF STOCK

### I. NORTH-EASTERN RHODESIA.

Our rapid passage through the country makes it impossible to do more than outline the distribution of domestic stock, and our task is rendered more difficult by the fact that in some districts we did not meet the local official, or were unable to visit his headquarters. We believe, however, that our information is exact, and in most cases one or other of us has visited the European-owned animals. We will discuss the distribution in magisterial divisions.

*East Loangwa.* In the vicinity of Fort Jameson the Administration has instituted a cattle farm, while settlers and missionaries around this capital all own stock. *Glossina morsitans* is practically limited to the North and North-West, and the natives (Angoni) of the neighbourhood keep cattle. There are a few horses or donkeys or mules in the district which are said to do well. We shall refer later to two outbreaks of trypanosomiasis which occurred in the healthy stock of Fort Jameson as the result of the importation of infected cattle.

The Native Commissioner of Petauki keeps some twenty head of Government animals for milking purposes.

*West Loangwa.* At Serenji some twenty head are kept by the Official, and the Livingstonia Mission there keep approximately the same number. The central part of this division is free from

*Glossina*, and we have heard no history of disease. There is only one settler, who has but recently acquired land and hired a few head of cattle from the Administration. We are not acquainted with any native-owned cattle in this division.

*Awemba.* It will be seen from the map that a large area of this district is tenanted by *Glossina morsitans*, and that only the North and North-West are considered free. The three Government posts of Mpika, Kasama and Luena each keep from twenty to thirty head of cattle for the Official's use, and at Kasama, donkeys and pigs were also seen. At Luena there have been no suspected cases of trypanosomiasis during the past two years, but previously a considerable number of these animals died there from 'fly,' which, it will be seen, encroaches all round. Mpika is also very close, and wandering *Gl. morsitans* have been taken on the station. There are no settlers in this division, but a European trader at Kasama owns a few head for milking. The mission stations of the Pères blancs d'Algiers at Chilabula, Chirui and Chilonga, each keep from thirty to sixty head, and none report the occurrence of disease, though *morsitans* were taken by us within four miles of the last named. It is said by natives that in past years the island of Chirui was joined to the mainland on the East side of Lake Bangueolo, and that tsetse were then existent during the dry season. This island is now somewhat densely populated, and so largely cultivated that nearly all timber has been cut down, and it is no longer connected with the mainland except by hardly fordable swamps of some miles in width. An Awemba chief, Luchembe of the Mpika division, keeps four head of cattle in the centre of *Gl. morsitans*. These animals are said to be the offspring of large herds brought in from raids on the northern tribes fifteen years ago. He has tried to introduce new animals since, but they have invariably died of disease. In the district around Kasama there are forty-five head of native-owned cattle, all of which live towards the border of the Tanganyika division. In Luena there are none. The natives on Lake Bangueolo keep relatively large flocks of sheep, which are said to do well locally; and most villages, even in the tsetse country, keep a few goats.

*Mweru and Luapula.* At Fort Rosebery, Madona, Kalungwisi and Chienji, we saw Government cattle used by the officials for milk. Deaths from 'fly' are said to have taken place at Fort Rosebery, and

the Government animals at Madona, which came from this station, were found to be infected. A trader near Sakontwi has had several deaths, while cattle owned by traders at Madona were suffering from trypanosomiasis. The stock seen at Kalungwisi and Chienji was healthy despite the proximity to both *Gl. morsitans* and *Gl. palpalis*. The mission stations on Lake Bangueolo, at Mbereshi and near the Johnstone Falls, have had no suspicious deaths. Kazembi, an important chief on the Luapula, owns a small herd which grazes on the large grass flats around his village, and Luari owns two or three head. Meri Meri lost a large herd a few years ago, and as noted in our previous report, considers *Tabanus* to have been responsible. Most villagers here keep goats, and a fair number also own sheep.

The distribution of *Gl. morsitans* in this division is not well defined, and we have had it reported to us from several areas where Europeans consider it absent. The importance of this division as a barrier to the spread of human trypanosomiasis makes it desirable that the exact distribution be mapped without delay. It was reported to us by chiefs in the South-East areas, that frequently during the windy season tsetse are carried over towards the South end of Lake Bangueolo from the Luera river, a distance of about twenty miles. At the time of our visit, March 1908, none were seen.

*North Loangwa.* Government cattle kept for the officials' use were seen at Fife and Chinsali, and those on the Government farm at Ikomba were also examined. A glance at the map will show the range of *Gl. morsitans* over the South of this division; the North, constituting part of the Tanganyika-Nyasa plateau, is free. On this plateau, from lake to lake, cattle are kept by natives, and only occasional sickness is reported. Trypanosomiasis was diagnosed in cattle at Chinsali by Yale Massey in 1905, and deaths have since occurred. At each of our visits (May and August, 1908) we detected cases, both in Government stock and on a settler's place at Scotsdale, three miles distant. Goats and sheep were also infected. As will be noted later, the Chinsali division affords striking evidence regarding the spread of *Gl. morsitans* within recent years.

A Government farm exists at Ikomba which was started in 1900 as a collecting centre for cattle then being purchased in German East Africa, and as a station for transport oxen, at that time in use on the Stevenson Road connecting Lakes Tanganyika



and Nyasa. At the present time it is used as a breeding station, and supplies cattle to the various district officials and replaces their losses in milch cows. Recently, unserviceable cows, males and young stock have been drafted in from these outlying stations, and among some such animals, stated to have come from Chinsali, we found trypanosomes. At the time of our examination of this farm there were 422 head of cattle, of which those that had been bred there were apparently in perfect health. In addition to the settler at Scotsdale, whose cattle, sheep and goats were all infected, there are two others, at Chunga and The Dell, situated within twenty miles of each other, and some twenty to thirty miles north of the present 'fly.' Cattle, some 500 head, at these two farms, looked well; Chunga, by reason of its large open grass flats, being particularly suited to stock. There has been no suspicion of endemic trypanosomiasis at these places, which were stocked originally from the healthy North. At the Livingstonia Mission near Fife some fifty head are kept, and though they were not in the best of condition we were unable to detect trypanosomes among them. On the plateau there are 1,708 head of native-owned cattle.

*Tanganyika.* The officials at Abercorn and Mporokoso keep cattle for their private use. The latter place was not visited by us, but the information given by the Native Commissioner does not suggest the existence of any acute trypanosomiasis. The disease has never been suspected in Abercorn, and we were unable to find trypanosomes in animals there; but a pig which was sent to us at our camp, fifty miles West, died of this disease soon after its arrival. The history of this animal will be given later, and we merely refer to it since it is possible that its infection may have taken place at Abercorn. Just prior to our visit some five deaths had taken place in a herd of nearly 600 head at a farm twenty-eight miles East of Abercorn, and we were able to see a case said to be similar to those which died. No trypanosomes or other blood parasites were detected, and from the clinical picture presented by this, and the histories furnished, we would support the possibility that a form of vegetable intoxication might be responsible. The existence of this sickness, however, is of interest, since on the route followed by one of us, each cattle-owning village between Kasama and Abercorn complained of disease or deaths. Owing to the absence of *Glossina*



particular attention was paid to these cases, and we purchased a sick animal in order to obtain a post-mortem. The only lesion which could be demonstrated was the presence of *D. hepaticum* in small numbers. Saving for the losses this year, native cattle throughout the division have done well, and tsetse (*Gl. palpalis*) only exist on the shores of Tanganyika and (*Gl. morsitans*) to the West of the division. Cattle are kept at the three stations of the London Missionary Society, Niamkolo, Kambole and Kawimbi. At the first-named there is no history of disease and our examinations were negative. At Kawimbi the cattle appear to have been affected by the same disease which occurred in the native cattle around; at our visit no trypanosomes could be found. Kambole is situated within a few miles of *Gl. morsitans*, though until 1905 cattle are reported to have done exceedingly well. In that year twenty head died, and since then the loss has been from four to six per annum. We found six animals in the herd of sixteen infected; their histories, so far as records are available, and the observations on the trypanosomes will be given later. In the Abercorn district there are approximately 1,200 head of native-owned cattle.

There are consequently between 8,000 and 9,000 head of cattle in Northern Rhodesia, localised, except for special purposes, to the district around Fort Jameson and to the Tanganyika-Nyasa plateau. Sheep are scattered over the territory, but are more extensively bred on the plateau and the district around Banguelo, and we are informed also in the valley of the Loangwa. Goats are distributed in most villages throughout. Dogs are kept in nearly every village; they are quite neglected, and live principally on offal. Wherever possible, Europeans keep a few, generally of English or colonial breed; and in these the mortality from trypanosomiasis is high, especially as they frequently accompany their masters on tour. Horses are found only at Fort Jameson and Fife; donkeys are met also at certain up-country posts and seem to have a high resistance to the local strains of trypanosomes.

## 2. NYASALAND.

We were unable to devote any attention to the question in Nyasaland, but the following summary, kindly furnished by the

Governor, Sir Alfred Sharpe, gives the approximate distribution of cattle in that territory. The relation to *Glossina* will be seen on the map:—

## CATTLE OWNED IN NYASALAND.

North Nyasa	By Europeans	426	By Natives	13,955
Mombera	"	146	"	22,000
West Nyasa	"	73	"	2,000
Marimba	"	205	"	764
Central Angoniland	"	1,923	"	860
South Nyasa	"	432	"	63
Upper Shire	"	873	"	885
West Shire	"	197	"	179
Zomba	"	1,634	"	10
Blantyre	"	3,891	"	176
Mlanje	"	557	"	nil
Ruo	"	123	"	150
Lower Shire	"	173	"	nil
			Indian	148
		10,653		41,190

The mineral resources of North-Eastern Rhodesia have not yet been developed; as a consequence, no roads exist and the movement of stock is confined. Prior to 1908, Southern Rhodesia, where the cattle had been destroyed by East Coast Fever, afforded a market to the breeders round Fort Jameson; and to speculators who travelled even into German East Africa to trade in cattle with the natives there. These latter drove their purchases by circuitous routes, avoiding tsetse so far as possible, down the Loangwa valley and through Portuguese territory; but the closure of Southern Rhodesia save through the ports of entry at Feira, for driven stock, and at Livingstone, for those sent by rail from North-Western Rhodesia, has practically stopped this movement, and the German frontier has also been closed owing to the rumoured existence of East Coast Fever in that territory.\* Traffic in stock between natives is probably rare, and is certainly quite local, and since the Administration have for some years wisely prohibited cattle trading between Europeans and natives, the only movements now taking place are between the few settlers, the stations of each mission society, or due to the

\* Government Gazette, North-Eastern Rhodesia, 1907-1908.

drafting of Government stock from the farms to district officials, or to settlers who hire cows for a term of years. Such movements will rarely exceed 100 miles. Transport is entirely by native porters, the few donkeys being used for riding in the district, and are only exceptionally taken any distance. Most of them have come originally from German East Africa.

### III. OCCURRENCE OF BITING FLIES

The barrier to the efficient and immediate development of this virgin country is unquestionably the wide distribution of trypanosomiasis and the transmitting flies.

1. *Glossina*. (a) *Glossina palpalis* is as yet only known from the river Luapula to the North of 12° South, around Lake Tanganyika and on some tributary rivers. It has not been incriminated for stock in Rhodesia, and cattle at Kalungwisi and Chienji are grazed close to its ranges. The distribution of this fly is given more in detail elsewhere (pp. 281-3).

(b) *Glossina morsitans* is found over the greater part of the territory; indeed, if the district of Fort Jameson, the Tanganyika-Nyasa plateau and the neighbourhood of Serenji were excluded, it would be difficult to assert its perpetual absence from any area of fifty miles square. The statement made by Sir Harry Johnston\* that this fly is not found in Nyasaland at an altitude of more than 3,000 feet does not obtain in Rhodesia, where the average height above sea level of the heavily infested Chinsali and Mpika districts is more than 4,000 feet, whilst they have been continually taken on the Machinga Hills to the West of the Loangwa, which approach closely to 5,000 feet, and a European crossing the Nyasa-Loangwa watershed East of Chinsali, which is of even greater altitude, states in a letter that he 'found the fly numerous right on the watershed on both sides of the border.' They are equally prevalent in the Loangwa valley (2-3,000 feet), and at less than 1,000 feet on the Shire river.

We were unable to make any personal observations as to the effect of season *per se* on the distribution of this fly; but the reports given us by residents indicate a lack of marked variations. The

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\* Johnston, Sir H. H., *British Central Africa*, London, 1897.



concomitant factor, wind, unquestionably influences the area of distribution, for during the monsoon the fly apparently disappears almost completely from the exposed places, such as the Loangwa Machingas already mentioned, and is carried over into normally clean country. We may instance in this connection the road which one of us followed at the end of the rainy season (March, 1908) from Kapata at the south end of Lake Bangueolo, via Kisengo and Kalasa to Sakontwi on the Luapula. No *Glossina* were encountered until reaching the river, but all the villagers en route were agreed that in the windy season of each year (June to September) they frequently catch tsetse, and they believe them to have been carried across from the perpetual zones on the Luera river, a distance of about twenty miles. This was supported by an Official; but it is to be admitted that the fact is susceptible of other interpretations.

The chief factor concerned in the distribution, so far as our observations can lead us to a conclusion, is the nature of the country and its vegetation.

The Native Commissioner at Sumbu on Lake Tanganyika has assured us that on more than one occasion he has taken *Glossina morsitans* near to that place, in the middle of a broad grass plain over half a mile in width, and that the fly rise from the isolated and insignificant shrubs which grow on the water-course there. This is the only evidence we have obtained of the occurrence of *morsitans* away from bush country; though the flats may be mentioned which extend from Sakontwi across the extreme corner of the Congo State towards Chitambo. Here, in August, 1907, one of us took this fly from around the tree-studded ant hills which crop up in the bare grass plains at intervals of one hundred to four hundred yards. To a greater degree, are they found in the park-like country which sometimes fringes the true bush country, and connects it with the larger of the open plains. Here the trees attain greater size and substance, but are set at such distances in the grass that the impression recalls a private park in England.

It is in the virgin forest or bush that *Glossina morsitans* takes a permanent abode. This type of vegetation covers the greater part of all watersheds and high-lying country, being broken only by the grassy 'dambos' which serve as drains. These dambos or open grass plains (vlei of the Dutch) commence as narrow strips of thirty



or forty yards in width, accompanying the streams, and like these unite together and sometimes open out as the large grass plains which accompany certain of the rivers. The timber for the most part is small, rarely exceeding thirty feet in height, and relatively open. The shade furnished by these trees is not always intense, but it is sufficient, when in full foliage, to afford comfort after the open. Owing to the almost annual fires, the branches are small and stumpy, and the undergrowth, excepting in a few areas, does not assume any great luxuriance or density and can be traversed with little or no discomfort. In the more densely inhabited regions (three to four inhabitants per square mile is the average) large tracts of this bush have been cut down about four feet from the ground and the land then dug for gardens. After about three years these are deserted, so that the vegetation of considerable areas on the inter-dambo ridges is of a more stunted character, but is apparently equally suited to the fly. The nearest approach to the dense tropical foliage which we met is found in the oases, termed *m'situ*, surrounding some springs; these are small areas rarely exceeding a few acres in extent, where the trees assume forest proportions, and are clothed with rubber vines and other creepers, and are interset with a luxuriant undergrowth; the soil is soft and richly vegetable, and the water is close to the surface. Although on arrival in the territory we were informed that these *m'situs* were tsetse areas par excellence, we have never seen *Gl. morsitans* in any of them, and it is possible that small *Tabanus* and certain *Haemaphysa*, which are numerous in most, may have been mistaken for *Glossina* by our informants. Regarding the nature of the soil we can say but little; the opinion is held by most Europeans and natives that *Glossina morsitans* avoids clays and swampy surfaces, and favours those of a friable or sandy nature. Our observations tend to bear this out, but the depth of the soil is very variable, being so shallow in many places as to permit of extensive out-crops of the subjacent rock.

There would appear to be no special desire for water on the part of the fly. In most parts of the central region with which we deal, many of the water-courses are dry for two or three months (August to October) at the end of the dry season, and one may have to travel in a direct line for over twenty miles in order to meet a permanent stream, which itself is little more than a trickle; and in instances could

be selected of districts where so far as is known no surface water exists in an area of twenty miles by twenty—400 square miles—and yet *Gl. morsitans* is permanently located there. This is perhaps exceptional, and it is unusual in the part of North-Eastern Rhodesia where we principally travelled, which is well watered by streams that are rarely so much as ten miles apart; but we may instance that to the South-West of N'dola in North-Western Rhodesia.

It is often stated as a fact that this fly will disappear from habitations erected in its haunts. This would appear to be true for North-Eastern Rhodesia, but it would be difficult to say whether it is due to the presence of man or to the inevitable clearing of the bush, with the consequent destruction of its natural haunts, necessitated by the building of many huts and the making of gardens. On many occasions we have taken *Gl. morsitans* within a few minutes of leaving a village, even one long established; and they are frequently located sufficiently close to follow natives daily.

In connection with 'following' flies, which we have watched being carried by natives for over half an hour without attempting to feed, it is an interesting fact, noted alike by the Administrator, Mr. Wallace, and ourselves, that although they may be so numerous as to constitute a perfect plague, most of them will quickly disappear when a camp is made in the midst of their haunts, and also even if the halt is but a temporary rest. On the other hand there are occasions recorded where our first capture on that day has been of flies apparently attracted by our arrival for lunch; these have, however, usually been in small numbers, ten or twelve in half an hour, and not in the swarms so often carried along, where that number could be captured with one sweep of the net, and where the lining of one's helmet and the back of one's neck would be almost hidden, if for a minute the energetic use of a fly switch were discontinued.

Local numerical variations are noticeable, and have frequently been noted by observant residents and by natives, even though the meteorological conditions were similar. On the few occasions when we have travelled more than once over a piece of fly country, it has been rare to see *Gl. morsitans* in the same numbers: on some such roads in the Mpika and Chinsali district they were taken plentifully in April and May, and were not seen in September or December; again at other spots they were taken in these latter months, and were

not seen at our first visit. Our first encounter with *Glossina morsitans* was near Broken Hill in July; not a single specimen could be found at the same time on the following afternoon, though the conditions of sun, wind and temperature were apparently identical.

It is unanimously stated by all who have known this territory for any time, that the area of distribution for *Gl. morsitans* is increasing. A noteworthy case is that of the Chinsali district.

The present Native Commissioner was one of the first Europeans in the country, and reached Chinsali in 1896. As one who had served in Southern Rhodesia previously, he was keenly alive to the importance of tsetse, and paid special attention to its occurrence, since he had been led, on coming into the country via Nyasa and the Tanganyika Plateau, to consider it one suitable for horses. At that time, 1896, from his own observations and from all reports he could receive from natives, *Glossina* was limited in that part of the country now forming his district to the neighbourhood of Itwa and the Chichera River. A map made in 1903, as the result of a special tour, shows the extent of the area then invaded, and to-day, with small exceptions, local and themselves uncertain, the whole district is under the influence of *Glossina morsitans*. The natives of this district are agreed in considering that the fly has enormously increased its ranges within recent years. This is reflected in the figures of native-owned cattle, which have decreased from 149 held by twenty-one owners in 1905, to eighteen owned by four men in 1907, and we were told by this official that in all probability there are now none (May, 1908). We were informed of two specific cases. In 1903 a chief settled in the district bringing with him healthy cattle and sheep. All died within two years, tsetse having encroached upon the land selected. A European on leaving for England in 1907 gave a drove of 13 pigs to a chief living on the fringe of the then fly-free country. Within three months these animals were all dead and the tsetse now surround his village.

There are several areas on the Tanganyika-Nyasa plateau and other supposed fly-free districts which correspond superficially with what we consider suitable *morsitans* country, and there appears no reason why it should not continue to extend. The existence of considerable open grass land in all these districts, however, will so intersect its distribution as to prohibit the infection of more than local strips which are in continuity with the permanently infected zones.

#### *Association with game.*

The question of the association of 'game' and *Gl. morsitans* is acutely discussed in Northern Rhodesia, and was ventilated in the *Field* towards the end of 1907. Owing to our temporary acquaintance with the country we are naturally not in a position to make emphatic pronouncements, but we may record here our observations on the diet of this fly. The game of North-Eastern Rhodesia with which we came in contact may be roughly grouped according to the



nature of the country they prefer. Most species at some time come to feed on to the grass dambos, which, as we have already said, are not permanently infested. The larger dambos are selected by some, while others, such as Kudu (*Strepsiceros kudu*), will rarely if ever leave the bush, or only occasionally appear on the fringe of the smallest clearings for a very short time. Rhinoceros (*R. bicornis*) and Buffalo (*Bos caffer*) chose the densest bush obtainable, and are commonly localised to those parts of the country affording this condition. In common with smaller game, they may come into the open in search of water or food, but they are rarely to be seen by sunlight except in the bush. (The local variations in the habit of the *Rhinoceros* which occurs on the plains of East Africa are interesting.) Opposed to these animals are the Sitatunga (*Tragelaphus spekei*) and the Lechwe (*Cobus lichi*), which never approach bush country, but live in the swamps and reeds, and the Sessaby (*Damaliscus lunatus*) and Puku (*Cobus vardonii*), which rarely penetrate into more than park-like country. Intermediate between these groups comes the majority of 'game'—Eland (*Taurotragus oryx*), Sable (*Hippotragus niger*), Roan (*Hippotragus equinus*), Zebra (*Equus burchelli*), Hartebeest (*Bubalis lichtensteinii*), and Waterbuck (*Cobus ellipsirymnus* and *C. defasa*), which spend the heat of the day in the bush, and come to the dambo to feed in the evenings and early mornings; some, as for example Waterbuck, Hartebeest, Roan and Zebra, perhaps favour more the open country, and in this agree with the smaller species, Reedbuck (*Cervicapra arundinum*) and Oribi (*Oribia scoparia*). The Bushbuck (*Tragelaphus scriptus*) is rarely found far from bush of a *m'situ* character, i.e., having water in the vicinity, and the duiker (*Cephalophus grimmi*) and M'pala (*Aepiceros melampus*) will seldom be seen feeding in the open. Pig, the wart-hog (*Phacochoerus aethiopicus*) and the bush variety (*Potamochoerus chaeropotamus*) are also found chiefly to the bush country and the edges of the dambos; and while Elephant may spend some time in and around water in the open, its food, leaves and bark of certain trees, is found in the timbered country and the garden clearings made therein.

We have never seen taken or suspected *Gl. morsitans* on animals grazing in the open, excepting when they were shot almost directly after emerging from the bush.



Reference has already been made to the observation that *Gl. morsitans*, even in its natural haunts, will quickly retreat from a person coming to a halt, although they may have been pestilent immediately prior to this. Our notes would indicate that they may recede from game in the same way, for upon the four occasions on which we came up to resting rhinoceri we could not detect any increase in the number of flies surrounding them—indeed, twice they appeared absent though we had captured them at the same spot on the previous day. The following case is of interest in this connection:—

One of us approached a herd of hartebeest against the wind, being badly annoyed by *morsitans* in so doing, and lay down on an ant-hill within thirty yards of the nearest animals. Four were lying down and four standing up, all apparently asleep, and judging from the 'spoor' they had been there some time. We remained on the ant-hill from 12.35 to 1.10 watching them carefully through prism glasses without being able to ascertain the occurrence of tsetse. Whether from habit or not we cannot say, but it is rare to see any of the tailed antelope keep that organ still for more than a few minutes, and in the present case the standing animals made periodic switches. For the last fifteen minutes of our watch we never saw or felt any tsetse on ourselves, though at least six were present when the ant-hill was reached, and we did not feel any bites. Two of these animals were then shot, but no fly were seen, though the rest of the herd did not at first move off more than 100 yards. On walking away we commenced to collect tsetse again within 200 yards.

Sir Alfred Sharpe and Mr. Harger have referred to districts in Nyasaland where game is plentiful and *morsitans* absent, and conversely, where *morsitans* abound and game excessively scanty. In North-Eastern Rhodesia the same disassociation is met with on localised areas. Speaking broadly, however, the best game country is in the Mpika, Chinsali and Kasama divisions, which for the most part are alternating bush and dambo affording ideal haunts for all varieties; and the concurrent existence of tsetse appears to us to be due to a preference for the same bush country. Certain of the Officials, all keen hunting men and observers, have substantiated this observation, and in reports have quoted instances in their particular districts which go to disprove any intimate connection.

It is well known that Mr. Selous has asserted that a peculiar affinity exists between the buffalo south of the Zambesi and the tsetse which occur there. In Northern Rhodesia there do not appear to be any grounds for this view, and men well versed in the country have denied it. Our own observations were made on the west side of Lake Bangweolo where tsetse are known to be found

periodically. In March, 1908, we followed the fresh spoor of three different herds, and on no occasion did we encounter any *Glossina*.

An answer to this question might be given if the diet of *Glossina* could be satisfactorily determined. It is, we believe, agreed that other blood-sucking Diptera—Culicidae, Tabanidae, and the related genera of the Muscidae, *Stomoxys*, *Haematobia* and *Lyperosia* can exist without blood; but it is commonly held, and Austen has recently emphasised his belief in this view, that *Glossina* demands blood and will not exist on plant juices. This writer bases his argument on the high specialisation of the genus; other writers, notably those from south of the Zambesi, on the rumoured inseparability of fly and 'game.' F. J. Jackson,\* Stordy,† and other observers in East Africa, Sir Alfred Sharpe and Harger in Nyasaland and Northern Rhodesia, have failed to notice any intimate connection, and our own work supported this. The extraordinary number of *Gl. morsitans* (almost incredible to one who has not been in their haunts) in many cases where 'game' is exceedingly rare, would appear to preclude the possibility that more than a small percentage could obtain a mammalian blood meal, at what one may suppose to be satisfying intervals. It is recognised that in captivity a tsetse must as a rule be fed *at least* every forty-eight hours; in nature it would often seem impossible for more than one or two per cent. to feed on blood, say, every six or ten days; in some cases, owing to the entire absence of any game indications, it would certainly look dubious if within a dry season the majority could get a blood meal.

Sir Alfred Sharpe in a private letter mentions that he had often been struck by the great preponderance of flies which on crushing apparently contained no blood.

We have never been able to keep captive flies alive for more than ninety-six hours after feeding, but up to that time there was invariable evidence of the meal. Certain variations in the rate of digestion were seen: in a few flies no corpuscles remained after thirty hours, in others they were seen intact after seventy-two. Following the disintegration of corpuscles the gut contents become granular and darker, and pigment grains are seen in the cells lining

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\*† F. J. Jackson and R. Stordy, vide Austen's *Monograph of tsetse flies*, London, 1903, pp. 295, 291.

the wall. With one or two exceptions this stage was recognisable after eighty-four hours. In these, and in those after ninety-six hours, the gut contents were fluid, only slightly pigmented and largely crystalline, but the cytoplasm of the lining cells is still deeply pigmented. In unfed flies—for present purposes we assume as unfed those showing no pigmentation—the cell contents are dull, finely granular, and free from pigmentation and vacuolisation; in those in which digestion is proceeding the cytoplasm becomes more refractile, less granular, vacuoles of varying sizes make their appearance, and the nucleus is more readily discernible.

It is consequently possible to state with certainty whether a given fly had obtained a meal of blood within four days of examination; it is probable that evidence of the meal remains for at least six days, and it is fair to suppose that a fly showing no signs of blood in its gut has not fed in this manner for five days.

On Lake Tanganyika (*Gl. palpalis*) and at Kambole (*Gl. morsitans*) we dissected nearly 400 freshly-caught flies, of which approximately 66 per cent. showed no signs of blood. Near Mpika 82 out of a total of 112 freshly-caught *Gl. morsitans* (79 per cent.) were free from all traces of haemoglobin, and of the twenty-three which showed such traces five had but recently fed—in three the sucking stomach contained large quantities—most probably from our caravan.

In three *Gl. palpalis* of which we have notes—prior to the first observation it possibly passed unnoticed—the cells lining the intestine from proventriculus to proctodaeum, were in the state we associate with active digestion, that is to say, there was refractility and vacuolisation of the cytoplasm, and the lumen was occupied by a clear fluid. It is impossible to state whether this was due to a pathological condition or to the digestion of a fluid which was not blood.

These observations, though unquestionably limited, would certainly point to *Glossina* as being capable of existence for considerable periods without blood, and possibly to their ability to feed on vegetable juices in its absence. We cannot neglect the additional evidence that 45 per cent. of our *Gl. palpalis* showed an intestinal infection with flagellates, and it is possible that some of



these acquired them through what Minchin\* has termed the 'contaminative' method.

As a result we are led to express the opinion that the distribution of *Gl. morsitans* is entirely dependent upon the nature of the country and its flora, the association with the fauna is largely fortuitous, and that a perpetual supply of mammalian blood is not imperative to its at least temporary existence.

#### *Relation to disease*

It has become almost an axiom that *Gl. morsitans* indicates trypanosomiasis; much further study is needed to decide this point. Wherever disease exists, whether positively diagnosed as a trypanosome infection or not, and this fly can be caught within five miles or perhaps more, even if it be but a solitary individual, the owner will remain convinced of its causal relationship. There are, however, certain instances which would indicate that this fly may exist within a short distance of cattle without producing any noticeable damage.

In a previous report† we noted an example on the River Kafue, and mentioned two villages (Chinyama and Chiwala) where cattle are, or have been kept, within a mile of permanently infested bush, and which unquestionably at some time have been bitten; indeed *morsitans* has been caught by a European feeding on these animals. In North-Eastern Rhodesia we were informed that cattle had been for some time grazed in the Loangwa Valley at a place where we took tsetse, and without any suspicion of infection resulting; and at Mpika and Luena, the natural haunts of the fly are so close that it is difficult to consider that these cattle have never been bitten. The same applies to the native cattle at Luchembe with *Gl. morsitans* all around, and to the Government-owned stock at Kalungwisi (*Gl. palpalis*) and Chienji. Still more striking examples are afforded by those herds which until recently were driven from German East Africa into Southern Rhodesia, approximately 1,000 miles. Of course, every precaution was taken to prevent contact with *Glossina*, and circuitous routes were followed to avoid them; but deaths were

\* Minchin. Proc. Roy. Soc. Series B., Vol. 79, No. 528.

† Montgomery, R. E., and Kinghorn, A. A Report on Trypanosomiasis of Domestic Stock in North-Western Rhodesia. Annals Trop. Med. and Parasitology, 1908, II, 2, pp. 97-132.



rare—we have no exact figures—despite a known infection in some of the animals. Mr. Morkel, the manager of the Government Cattle Farm at Ikomba, gave us the following particulars, which afford valuable evidence regarding the effects of *Gl. morsitans* on cattle in one part of this territory.

He travelled with a mob of 800 head of Government cattle from Ikomba to Fort Jameson, starting in November, 1907. The route followed may be seen on the map. *Glossina morsitans* was first encountered on December 25th near Kabomba and it continued practically all the way down the East side of the Loangwa to Chinundu, forty miles from Fort Jameson, which was reached towards the end of March, 1908. At Msikini, where a week was spent, fly was very thick, and at Chipandwi, with fly all round the village, these cattle were quarantined for six weeks. Actually twelve weeks were spent in permanent haunts of *Gl. morsitans*; the cattle marched over 400 miles—roughly 200 of which was in 'fly'—and did so in the middle of the rainy season, with daily storms and several rivers which had to be swum across, and they arrived in Fort Jameson with an actual loss of only eight. Of those which arrived eighteen were believed to be infected (the method of examination adopted in this herd is not known), but we were informed by the Veterinary Officer that after a rest near Fort Jameson the mob was sent on to its destination in Southern Rhodesia and was received there without further loss. It is to be noted that all three dogs which accompanied Mr. Morkel died.

It is assumed as a result of Bruce's work in 1896 that animals ranking as game, constitute the reservoir from which *Glossina* abstract infection, that they are what Minchin\* and Woodcock† term 'natural' hosts for the trypanosomes.

What proportion of game is infected cannot yet be estimated; a very small percentage apparently shows peripheral trypanosomes, and it is extremely difficult to carry animals susceptible to inoculation, especially since small laboratory animals would fail to demonstrate at least two organisms pathogenic to domestic stock, *T. vivax* (*T. cazalboui*) and *Tr. nanum*. The number of head showing trypanosomes in the peripheral circulation is certainly small; Bruce‡ failed to demonstrate them in Zululand; Dutton, Todd and Kinghorn§ record three positive findings in twenty-two; and we have found them in only two out of 158 direct examinations. It will be detailed later that inoculations were made from these cases and from a wart-hog and a buffalo shot near our camp, in every case without result. Bruce's inoculations at Ubombo showed approximately

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\* Minchin. Proc. Roy. Soc. Series B., Vol. 79, No. 528.

† Woodcock. Art. Haemoflagellata in System of Zoology, Vol. 1, Fasc. 1. Edited by Ray Lankester. London, 1909.

‡ Bruce. Further Report on Nagana. London, 1897.

§ Dutton, Todd and Kinghorn. Annals Trop. Med. and Parasit., Vol. I., No. 2.

25 per cent. of the local fauna to be infected; with such a high proportion the *Glossina* should also show a correspondingly high percentage of infectivity, and in Zululand this apparently obtained, for no failures are recorded. That this high ratio of infected tsetse is not universal, is we think shown by the manner in which the cattle already mentioned have been exposed to their attacks for considerable periods without any untoward effects; unless it be assumed that a very latent or chronic infection had resulted.

We have records of feeding fifty-three freshly-caught *Glossina morsitans* on a dog (22), a guinea-pig (25) and a white rat (6), none of which became infected; and no natural infections have been recorded by workers of the Sleeping Sickness Commission,\* who used large numbers of freshly-caught *Gl. pallidipes* and *Gl. fusca* at Nairobi. The suggestion was then made that possibly these flies had lost their infectivity during the period elapsing between capture at Kibwezi and feeding at Nairobi, but if Kleine's recent observations† are to be substantiated it must be inferred that they were non-infective at the time of capture.

The Loangwa valley down which Mr. Morkel travelled is one of the best shooting districts in North-Eastern Rhodesia, being very rich in game of all local species; whilst the Kafue river, on which were situated the European farm and Chinyama's village, where no cattle mortality occurs, is one of the richest shooting grounds in North-Western Rhodesia, and, as previously reported, game of various species has been seen grazing with the stock.

Quite dissimilar are the results in the transport cattle taken in 1907 from Broken Hill to Kansanshi, where all the 108 head used on this 250 mile journey died within two months, and all the 42 head sent between Broken Hill and Ndola also succumbed. This result also occurred, on the same Ndola road, to the railway survey party, who lost all their animals in 1905-6 and had to abandon their waggons, and to Mr. George Grey's party proceeding from Kalomo to Kansanshi in 1902.

Reference has been made in our previous report to two experimental animals taken by us between Mwomboshi and Broken Hill which became infected after three *Gl. morsitans* were seen to

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\* Reports of Sleeping Sickness Commission, No. V, p. 45.

† Kleine. Deut. med. Woch., 1909, pp. 469-470.

feed. In a supplementary note at the end of this report we shall draw attention to the possibility that they derived infection after arrival at our camp.

It is, of course, easy to explain the discrepancies in the evidence quoted as being due to lack of infectivity in the tsetse or the game, or that the trypanosome infection was so mild as to escape clinical detection, but it appears to us that this is a confession of ignorance on the most essential points, upon a knowledge of which improved prophylactic measures might be adopted; and when we add to this the fact that all game experimentally inoculated\*—zebra and zebra-hybrids, a springbok, jackals, most monkeys, rats, &c.—became infected, it must be admitted that a thorough re-investigation of the relationship between game, tsetse and disease should be undertaken.

Are other biting flies not implicated in this disease? Tabanidae (*Tabanus* and *Haematapota*) and *Stomoxys* are irregularly distributed over the whole country we travelled; but in much smaller number than *Glossina*. *Chrysops* is uncommon and *Pangonia* was only taken at three places, each roughly twenty miles apart at points of a triangle—Chunga, Dell Farm, and close to Mirwangi Village. We never heard of it elsewhere. *Hippobosca* was not seen by us in Northern-Eastern Rhodesia, but Neave has taken them in the Lovu Valley.

In a previous paper† reference was made to *Tabanus* acting as transmitting agent. A second native has made a similar accusation, asserting that this fly was responsible for many deaths in cattle at Kota-Kota in 1903. Europeans and other natives have never had occasion to incriminate Tabanidae and use the well-worn argument that they exist where there is no disease. Only one exception may be made for the case of a European settler who has blamed *Haematapota* for the death of some donkeys. We have not personally met this settler and have no first-hand data.

The case against *Stomoxys* is different. We have concluded that in an epidemic at Broken Hill this played an active part in extending transmission to those cattle which had not recently at least been in

\* Vide Jakimoff. Cent. f. Bakt. i Orig., Vol. XXXVII, p. 668. Kanthack, Durham and Blandford. Proc. Roy. Soc., 1898, LXIV, p. 100. Martini. Deut. med. Woch., 1903, pp. 573-575. Grothusen. Arch. f. Schiff- u. Tropenhyg., 1903, VII, p. 387.

† Montgomery and Kinghorn. Annals Trop. Med. and Parasit., Vol. II, No. 2.



'*morsitans* country.' Since then we have learnt of two outbreaks of trypanosomiasis in cattle near Fort Jameson, which, as already noted, is tsetse-free and a cattle raising country. The following is abstracted from the report of Mr. Lane, the Veterinary Officer to the Administration\* :—

In March of this year (1908) a few deaths in cattle occurred on a farm twenty-five miles from Fort Jameson, considered at first by the owner to be due to brutality on the part of the herd-boys. Later it was considered to be Biliary fever, and finally the owner attributed the recurrent deaths to liver fluke. In a blood smear, sent in at this time, trypanosomes were found. A visit to the farm disclosed the fact that some Government-owned stock lent to this settler, and which had never before been issued and were then undoubtedly 'clean,' were amongst the infected. This settler admits buying cattle from natives, and I fear that at the time he bought this stock, although showing no signs of disease, they had trypanosomes, and I think a blood-sucking fly had conveyed them to the healthy ones. No 'fly' can be found at this farm, and the owner denies that his animals have ever left. I have little doubt in my own mind that a blood-sucking fly other than the *Glossina morsitans* can convey the disease. It is possible that small numbers of 'fly' may have rapidly crossed the farm, but it is difficult to believe this after hearing the emphatic statements of the owner and his boys who must be cognisant with the tsetse. Up to the present between thirty and forty animals have died.

A second outbreak occurred on a part of the Government Farm thirteen miles from Fort Jameson and the Broken Hill road. Eight head of cattle were sent from Petauke in January, 1908, by the Native Commissioner, who recorded that they all were then in good condition. When they arrived they were naturally, after travelling for over a hundred miles, somewhat poor in condition. These animals were herded with other Government stock at a village on the farm. A month after their arrival they were reported sick, and blood smears showed trypanosomes. Five of these animals have already died. At the beginning of April, I was again called by the foreman of the Government farm, as he reported other animals were sick. These animals were thin and unthrifty in condition, and blood smears showed they were also suffering from trypanosomiasis. The serious side of this outbreak is that some of the animals had not been issued to farmers for a number of years, and others had been on the farm for five or six years. This discovery again points to the fact that a blood-sucking creature other than *Glossina morsitans* can convey the disease in cattle. I must add that every pains have been taken to find out if tsetse fly had visited this district, and from all sources I have got a negative reply.

Mr. Lane concludes by observing that flies 'very similar to, if not the ordinary house-fly,' were present in 'unusually large numbers' and 'were constant suckers of blood of these animals,' and proceeds to discuss the possibilities of mechanical transmission under these conditions.

We have, in North-Eastern Rhodesia, met a European† who has been twenty-four years in tsetse infected districts and who had previously never suspected any fly but these. As a result of his own

\* Kindly forwarded by the Administrator, Fort Jameson.

† Mr. G. Pirie.



observations in January, 1908, he concluded that *Stomoxys* was responsible for the death of two dogs, showing all symptoms of trypanosomiasis, which took place in reported fly-free country.

On a farm—Scotsdale—near Chinsali, we found cattle, sheep and goats naturally infected. One of the infected cattle was a calf four months old. The owner's son said that the cattle habitually grazed on the grass flats just below the farm, but that the calf remained in the homestead or in the gardens around. *Gl. morsitans* does not occur within two miles of the river, across which the calf had certainly never gone. The adult cattle, four, all that remained of twenty-eight, are believed to have been infected on the way to the farm six months before. *Stomoxys* was pestilent in the cattle kraal.

The trypanosome at Broken Hill was *T. dimorphon* and that at Fort Jameson was morphologically of the same type and was pathogenic to dogs.\* That at Scotsdale, recalls *T. nanum* in so much that morphologically it shows forms similar to the tadpoles of *T. dimorphon*, and that all inoculations in guinea-pigs and rats have been negative.

While investigating an outbreak of trypanosomiasis in Portuguese East Africa where *Glossina* is said to be unknown, Theiler† has also met with an organism responding to the tadpoles of *T. dimorphon*. Here again other biting flies must be incriminated.

In the face of this evidence it might at first glance appear strange that no outbreaks of trypanosomiasis have occurred in Southern Rhodesia as a result of the importation from the North, of cattle in whose blood trypanosomes have been found on arrival.‡ But due consideration will show that while there is no proof that cases have not occurred there, the chances would be against a free transmission. Owing to the dearth of local stock, due to losses from East Coast Fever, most of the imported animals were intended for slaughter on arrival; further, the quarantine regulations being in force, movement of all stock was prohibited or rigorously controlled. In addition to these factors, which of themselves would inhibit the transmission of

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\* Private communication of the Veterinary Officer, Fort Jameson.

† Theiler, A. Bull. Soc. Path. Exot., T. II, No. 1.

‡ See Report of Pan-African Veterinary Conference, 1909. Department of Agriculture, Pretoria.

disease, we have no evidence as to the geographical or numerical distribution of *Stomoxys* south of the Zambesi. Again, it is thought by most who have not studied the question that trypanosomiasis must occur in an epidemic form; they do not appreciate the fact that in most countries where the disease exists in the absence of *Glossina*, as for example in India, the greatest mortality occurs as a result of isolated cases and that anything corresponding to an epidemic is relatively rare, and when occurring is due to the circumstances under which the animals were kept. Such isolated cases, where the disease is not suspected, and due to trypanosomes of diverse animal reactions, are most readily overlooked, and the death would probably be ascribed to the commonest complaint. South of the Zambesi the name gall-sickness covers a multitude of diseases.

#### IV. THE DISEASE IN STOCK

We have met with the disease naturally in cattle, sheep, goats, dogs and a pig; all examinations of donkeys, nine in number, have been negative.

Concerning the susceptibility of *Donkeys*, we would recall that at Broken Hill two were inoculated, and one became infected with *T. dimorphon*. On December 28th, fourteen months after inoculation, he was still at work, and had exchanged hands at an enhanced price owing to his improved condition. The donkey inoculated with *T. vivax* did not show organisms during the period of observation, and five months later he was taken back to Chinsali by his original owner. We examined him there on two occasions with negative results, as also were those made on three other donkeys living close to *Gl. morsitans* and being worked therein, and sleeping in a kraal with trypanosome-infected cattle. The donkeys with which we came in contact apparently possess a high degree of resistance to *T. dimorphon*, *T. vivax* and a trypanosome allied to *T. nanum*. Beyond the mention of donkeys dying after the bites of *Haematapota*, a case already referred to, we have received no information as to their behaviour in other districts to which they may have been taken.

Trypanosomiasis in *Cattle* would appear to vary in intensity very considerably. The severe outbreaks of acute disease at Broken Hill and Fort Jameson contrast markedly with the latent infection at

Chinsali, which has certainly been in existence since 1905, and which normally takes its toll of but two or three per annum.

As will be expected, the symptoms vary with the nature of the infection: from the acute febrile condition of a week or two's duration to the emaciated hairless skeleton, which has presumably taken at least a year to produce. In the absence of any positive diagnosis, recovery may be disputed; but certain owners are emphatic that animals with a history of exposure, and all clinical symptoms of trypanosomiasis, have recovered, and we have examined such without seeing trypanosomes. Neither can the post-mortem appearance be regarded as constant. The enlarged spleen and haemorrhagic glands of the acute case; the oedematous tissues and accumulation of fluid in the body cavities; and the dry, fatless muscles, the pale tissues and organs with slight oedema of the lymphatic glands—the only moisture present—are according to the observation of ourselves and of others, all met with.

Further work on the nature of the trypanosomes encountered in the territory, and their geographical distribution, is required before the questions of insusceptibility, immunity and recovery can be logically discussed.

*Goats* and *Sheep* are regarded alike by Europeans and natives as immune. On *prima facie* grounds the disease in them is therefore chronic. We have seen animals three and a half months after the diagnosis of the natural disease in as good condition as at first, and not suspected of sickness by observant owners.

Twenty-three goats and sheep were successfully inoculated with various strains; the only failures, three, being at Broken Hill with *T. vivax* (gland puncture was not resorted to).

Speaking broadly, trypanosomes were generally visible in the peripheral blood, both in naturally and experimentally infected animals, and being apparently in good health, it appears to us that they may act as reservoirs par excellence for the virus, and by trade and movement may become dangerous potential disseminators of the disease.

In *Dogs* the disease, when fatal, appears to be somewhat acute, and accompanied by intermittent fever, progressive debility, opacity of the cornea, and frequently by nervous symptoms. It would seem,



however, that dogs will not infrequently recover from what is clinically a trypanosomiasis.

We have notes given by the owners\* of two dogs which travelled from Broken Hill to Abercorn and which were healthy on starting. The only tsetse actually seen were encountered about the middle of April, and were noticed to have settled on these dogs. They arrived in Abercorn the end of April, and a fortnight later, a month after meeting *Gl. morsitans*, both lost flesh, became listless and dull, and had uncertain appetite. This continued for about three weeks, after which they gradually picked up, and at several blood and gland examinations between June and November no trypanosomes were ever seen, and the dogs are both quite healthy.

Two other dogs travelled from Sumbu to Mporokoso, and from four to eight weeks after the journey lost all condition, became veritable skeletons, and were covered with body sores. A third dog which accompanied one of these had to be shot. In one case there was total 'blindness' and opacity of the cornea. They commenced to improve after about two months' sickness but regained their condition very slowly. We examined their blood and glands on several occasions between June and November without finding trypanosomes, though they were still thin.

As indicating the course trypanosomiasis may take in a dog, we may here mention the case of a wire-haired fox terrier which accompanied one of us from Broken Hill and back to the railway nineteen months later.

This dog encountered fly in August, 1907, and was thereafter as much in it as out, and we have on many occasions removed gorged *Gl. morsitans* from him. From December to February he was somewhat less active than usual, and towards the end of that month rapidly lost condition, temperature was elevated and spleen enlarged, but we were unable to demonstrate trypanosomes in the blood. He rapidly recovered, but again fell sick in October, 1908, and trypanosomes were then seen. In November his condition was precarious and a fatal termination almost daily expected. From the night of November 24th he rallied and retained his improvement up to arrival in Pretoria in the middle of January, when, despite his lack of bodily condition from the long journey, he was mentally active. Trypanosomes were not seen in blood and gland examinations between November 13th and January 20th, but death took place from this disease shortly after our departure (Dr. Theiler's letter of April 26th).

We have only seen the disease in one *Pig*; though it has been recorded to us from Chinsali and was then apparently acute. We are unable to trace the infection of this pig, which had been at our camp for three weeks in apparent health. Death took place after a visible sickness of only six hours' duration, with trypanosomes swarming in the blood and post-mortem changes indicating an intensely acute disease. These animals are quite local in distribution and of little economic importance.

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\* Messrs. H. C. Marshall and J. Deacon.



## V. PROPHYLACTIC MEASURES RECOMMENDED

### *Prevention.*

In a country like Northern Rhodesia we are faced by the interests of two sections of the community—those who wish to breed healthy stock in suitable localities and those who for commercial reasons find it necessary or desirable to keep or expose their animals in localities where the potentialities of the disease—infective tsetse—exist.

For this latter class there is little to be said: the owner is cognisant that all his animals may die, and if he is engaged in hired transport his charges are sufficient to indemnify him. These high rates—we have heard of £60 per ton being paid for transport over 250 miles of road—act most detrimentally to the interests of commerce in a young country, but in the light of our present knowledge there appears no solution. Theoretically the removal of the tsetse, or the source from which they obtain the infection, would bring about the desired result. In practice the former is, as yet, impossible, though according to the theory prevalent amongst those who have been in South Africa, the destruction of the game, which may also be considered the main source from which infection is derived, would be followed by the disappearance of *Glossina*. A serious attempt was recently made near Fort Jameson by men acutely interested in the result, to destroy or drive away the game from an approved area. A successful termination to the experiment would in all likelihood have meant the adoption by the Administration of this means of clearing all areas where cattle movement was desirable, and it may reasonably be supposed, therefore, that every effort was made in order to obtain this substantial assistance. We are informed\* that the agitators confessed their inability to make any noticeable impression, and realised the utter impossibility of removing the vast herds of game which tenant the country.

We are more concerned in safeguarding the interests of those fly-free districts where trypanosomiasis is not yet known to be endemic; that is to say the areas of North-Eastern Rhodesia referred to already as Fort Jameson, the Tanganyika-Nyasa Plateau and Serenji; the areas south of the River Kafue, the Barotse and Masha-

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\* Private communication from Fort Jameson.

kalumbue countries, and other localised parts in North-Western Rhodesia, together with the healthy parts of British South Africa.

We fully realise the issue at stake, its predominant importance, and the difficulties to be contended against, especially the local or individual considerations which must be respected. In brief, the suggestions offered are set out below, and, since the native does but little cattle trading except with Europeans, and this is now prohibited, they are drawn up as regards bona-fide settlers and stock owners.

1. The first essential is to obtain maps with the distribution of all species of *Glossina* and the occurrence of the disease, made to the satisfaction of the local officials in concert with representatives of the local stock owners. A central committee in each territory, which should include the Chief Veterinary Officer and delegates of the stock-owning community, would proceed to divide the territory into the two heads 'Infective' and 'Clean.'

Under Infective would be marked all areas permanently inhabited by *Glossina* of any species and a zone of not less than five miles surrounding, and all places without, if any, where the disease may be considered endemic.

Clean would, in general, be 'fly free' areas.

2. All equines and bovines resident within the infective areas, as at outlying Government posts, farms, mines and missions, should be branded in a distinctive manner, and a register of all such animals, containing a full description and all marks of identification, should be kept, and all equines and bovines entering an infective area from without should be similarly branded and registered.

3. As soon as possible an inspection should be made of all stock domiciled within the 'clean' areas and a certificate issued by the Chief Veterinary Officer to stock owners who have branded or otherwise identified their animals in a register to the satisfaction of the Inspecting Officer, and whose animals are free from this disease. Should a case of trypanosomiasis be detected, that area should be regarded as temporarily infective and should be placed in quarantine. By stock is here meant all equines, bovines, sheep, goats, swine and dogs, and should also include any wild mammal kept in captivity which at the discretion of the Veterinary Officer might be held to constitute a danger.

4. No movement of any animal should be permitted from an infective to a clean area save with the sanction of a Veterinary Officer, and on no account should any such animal be allowed to approach within 400 yards of clean stock.

5. All premises, herds or flocks of a clean area in which a case of trypanosomiasis has been found, and all stock entering from an infective area should be placed in quarantine, and not allowed to come within 400 yards of any other stock.

6. Quarantine should be maintained for at least three months after entry, or, in 'contact stock,' after the diagnosis of the last infected case. All animals therein should be subjected to repeated blood examinations, gland puncture or sub-inoculation, and at the expiration of that term they should be carefully registered and liberated on a veterinary certificate.

7. Every case of trypanosomiasis in a clean area should be immediately destroyed; or in certain approved instances placed in a special segregation camp situated at least 400 yards from all other stock.

8. These suggestions should be so amended as to permit of respect being paid to the legitimate requirements of localities or individuals; as, for example, in the case of dogs, which might, after inspection and registration, be permitted to remain with, and at the responsibility of their owners; and certain latitude might be permitted in the case of cattle, sheep and goats intended for slaughter.

The adoption of these suggestions would, we think, effectually prevent the introduction of trypanosomiasis into the districts at present believed to be free from it; and if the definitions of infective and clean areas were made in consultation with the Governments of South African Colonies and the clean areas proved to their satisfaction to be free from disease, there could be no objection to a proposal that legitimate trade, subject only to the ordinary restrictions, should not proceed uninterruptedly with the clean areas South of the Zambesi. At present, with the existing conditions largely unknown, Northern Rhodesia as a whole is regarded with a certain degree of suspicion, a punishment which reacts with disproportionate severity upon the large cattle breeders established in healthy districts.

A careful examination of these suggestions will show they do no more than protect the healthy, and will in no way interfere with



existing trade, but are designed rather with the object of organising this, to the best individual and collective advantage.

Besides legislative interference, however, it is to be urged that stock owners should be made acquainted with the full etiology of trypanosomiasis as at present known, and every effort made to discourage the views previously held that the tsetse alone can give infection and that goats and sheep are not attacked. These animals, and donkeys, when indiscriminately moved, may, owing to their apparent health be of greater danger to cattle; and legislation should carefully deal with them, for their identity and registration will be difficult.

## VI. THE TRYPANOSOMES ENCOUNTERED

### (a) IN NATURALLY INFECTED RUMINANTS

#### I. 'Scotsdale.'

Origin: At our first examinations, May, 1908, of the stock on a farm known as Scotsdale, some three miles from Chinsali, we found four cattle out of five, four sheep in eleven, and one goat in four to show trypanosomes. These cattle had been brought from the Loangwa valley, and it is possible they were infected on the road, for *Gl. morsitans* exists plentifully on that side of the farm. On the other hand, a calf born on the place and not exposed to this fly was infected—a case which suggests the operation of a local genus such as *Stomoxys*, which was very prevalent in the kraal occupied by all the stock. Owing to the owner's absence we were unable to learn much regarding the natural disease in these animals, but it would appear, from what was adduced, that it passes unnoticed in sheep and goats, and lasts at least six months in most bovines. On our second visit, four months later (August-September), we found that only one cow had died in the interim; the other bovines, excepting the calf, did not appear much thinner; the sheep and goats still retained their normal condition.

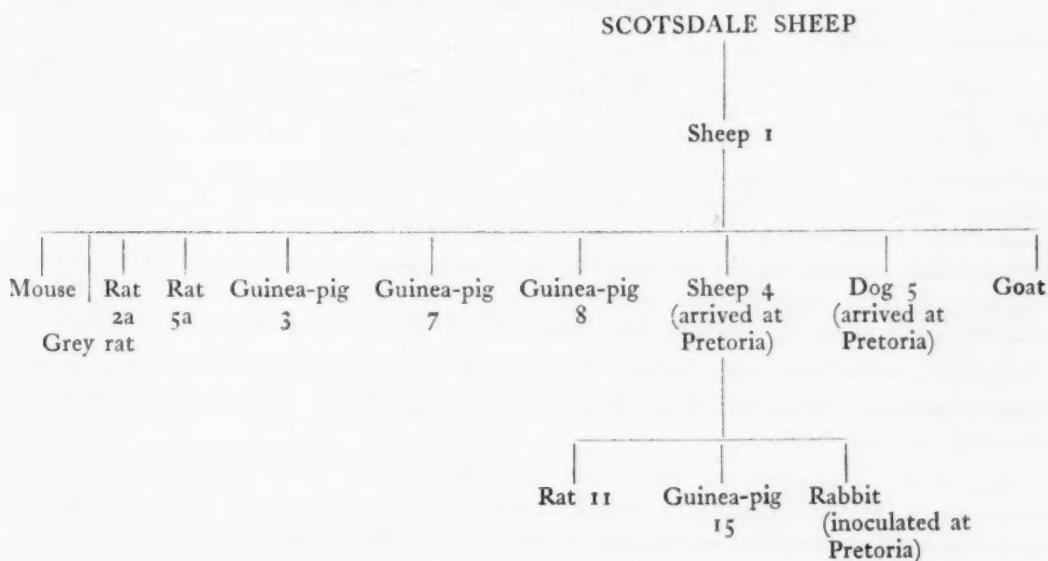
In May, two rats were inoculated intraperitoneally with 0.5 c.cm. and 1.0 c.cm., respectively, of citrated blood from two of these cows. They did not become infected. On our subsequent visit two rats and two guinea-pigs were given intraperitoneal injections of from 3.0 to 10.0 c.cm. of blood containing trypanosomes (in one



case, two. to a field) from three bovines, without result. The owner had very kindly written, giving us permission to take away an infected sheep or goat that the strain might be carried to our camp at Kambole; and a sheep which showed trypanosomes at both visits was selected. We take this opportunity of thanking Mr. Yule for his consideration, without which even the little possible could not have been done.

This sheep was carried in an improvised hammock for ten days, when it was found to have lost its condition and become very weak. On the twelfth it was *in extremis* and was destroyed (September 12th), and 5.0 c.cm. of blood containing trypanosomes one to a field was injected into a Sheep No. 1, which had been purchased in a healthy village and been examined by blood and gland puncture on three occasions with negative results. This sheep, which became infected about the eighth day, reached Kambole on September 21st, and served as the source of the strain.

The following is the genealogy of this strain:—



### Experimental

The inoculations here described are those carried out with the original Scotsdale sheep, or its passage through Sheep 1.

**RATS.**—Two white rats received, the one, No. 2a, 6.0 c.cm. (24.9.08), the other, No. 5a, 5.0 c.cm. (22.10.08) intraperitoneally, of blood containing numerous trypanosomes from Sheep 1. One white rat, No. 11, received 10.0 c.cm. (25.10.08) intraperitoneally from Sheep 4 (inoculated from Sheep 1) trypanosomes one to a field. None of these rats became infected.

GUINEA-PIGS.—Three guinea-pigs were inoculated intraperitoneally from Sheep 1; No. 3, 6.0 c.cm; No. 7, 30.0 c.cm; No. 8, 20.0 c.cm; and one, No. 15, with 15.0 c.cm. from Sheep 4. None of these became infected.

A WILD MOUSE (Sp. ?) received 0.4 c.cm. subcutaneously, and a GREY RAT 3.0 c.cm. intraperitoneally from Sheep 1. The mouse escaped on the 18th day, but had not up till then shown trypanosomes. The rat was under observation for two months and trypanosomes were never seen.

RABBIT.—We were unable to utilise rabbits in Rhodesia, but on arrival in Pretoria a rabbit was inoculated intraperitoneally with 5.0 c.cm. citrated blood of Sheep 4. Trypanosomes appeared on the seventh day. Further observations are necessary to determine the constancy of this reaction in rabbits, but there is no reason to consider that any contamination of this strain had been effected during the journey.

DOG 5.—An adult 'Kaffir' dog was inoculated intraperitoneally on October 22nd, 1908, with 10.0 c.cm. citrated blood of Sheep 1. The temperature became irregular from the eighth day and reached 103.6 on the evening of the thirteenth. During the daily examinations to November 15th no trypanosomes were ever seen either in the peripheral blood or on gland puncture, and the dog remained healthy in appearance. On November 27th one trypanosome was seen in a  $\frac{3}{4}$ -inch cover-glass preparation. From that date to January 20th, at Pretoria, they were not again seen.

SHEEP.—A naturally infected sheep was presented to the Expedition by Mr. J. B. Yule, of Scotsdale, and constituted the origin of this strain. It was in fair condition at the time of leaving Scotsdale, but rapidly became debilitated with travelling, though carried in a hammock, and was destroyed, when *in extremis*, on September 12th.

SHEEP 1.—September 12th, 1908, inoculated subcutaneously with 5.0 c.cm. blood direct from the original animal. Organisms appeared about the eighth day. It was carefully carried to our camp at Kambole and on arrival there, September 22nd, showed trypanosomes. The temperature was elevated and irregular, and did not show the striking picture manifested by a chart of the Broken Hill *T. dimorphon*. Organisms were present daily up to September 30th and were then absent for ten consecutive days, reappearing on October 11th. From October 1st the temperature assumed a much more irregular type which persisted to October 23rd when the animal died of septic pleuro-pneumonia.

SHEEP 4.—October 10th, 1908. Inoculated intraperitoneally with 30.0 c.cm. citrated blood of Sheep 1. This large dose was given as trypanosomes had not been seen for ten days.

Organisms appeared on the sixth day and have been almost constantly present since, though always in small numbers, it being rare to see so many as one to the field, more commonly one to five or ten fields being noted.

The temperature showed little abnormality, and no paroxysmal tendency.

This sheep ('long-eared') was carried to Pretoria where it is under the care of Dr. Theiler who, under date of April 26th, writes that it is still alive.

GOAT 2.—Inoculated subcutaneously with 5.0 c.cm. blood of Sheep 1, October 22nd. The temperature suddenly rose to 105.7° the evening of the tenth day, and trypanosomes were present the following morning, and were seen daily, with two exceptions, during the twelve days this animal was observed. The temperature tended to assume the type noted with Broken Hill *dimorphon*, being elevated to 106° and 107° about every second day. It was destroyed, having shown itself susceptible on the twenty-second day.

The following inoculations were made from other animals at 'Scotsdale'; it is believed that the trypanosome is the same.

FROM COW A.

May, 1908: White rat 0.5 c.cm. intraperitoneally.

August, 1908: White rat 3.0 c.cm., Guinea-pig 5.0 c.cm., intraperitoneally.

No infection resulted.

FROM COW B.

May, 1908: White rat 1.0 c.cm. intraperitoneally.

August, 1908: White rat 5.0 c.cm. intraperitoneally.

No infection resulted.

FROM CALF.

August, 1908: Guinea-pig 10.0 c.cm. intraperitoneally.

No infection resulted.

At our first visit to Chinsali, the position of which with regard to *Gl. morsitans* has already been discussed, two cows in a herd of nine adults were found infected. No reliable history regarding these animals could be obtained owing to a change of Native Commissioner and the fact that no individual records are kept; but it is possible that these animals came from Mirongo three months prior to our visit. *Gl. morsitans* exists on that road.

These same animals were again seen at Ikomba Government Farm in August and September and were still infected. The drafting of these cases to a healthy area is a source of danger which could be avoided; it shows also the desirability of branding and identifying all stock in these areas, since it was only by our own notes and enquiries that they were proved to be the same cattle, and not cases of trypanosomiasis occurring spontaneously on this Farm.

The morphology of this trypanosome is identical with that at Scotsdale, and we consider it probable that they are the same species.

The following inoculations were made at Chinsali:—

Cow A.

May, 1908: White rat 0.5 c.cm. intraperitoneally, Guinea-pig 2.0 c.cm. intraperitoneally.

Cow C.

August, 1908: White rat 2.0 c.cm. intraperitoneally, Guinea-pig 6.0 c.cm. intraperitoneally.

In no case did infection result.

At Kasama, in May, we found a sheep, which had arrived from Lake Bangweolo four days previously, showing trypanosomes which are morphologically identical with those occurring in the Scotsdale



GUINEA-PIGS.—Three guinea-pigs were inoculated intraperitoneally from Sheep 1; No. 3, 6.0 c.cm; No. 7, 30.0 c.cm; No. 8, 20.0 c.cm; and one, No. 15, with 15.0 c.cm. from Sheep 4. None of these became infected.

A WILD MOUSE (Sp. ?) received 0.4 c.cm. subcutaneously, and a GREY RAT 3.0 c.cm. intraperitoneally from Sheep 1. The mouse escaped on the 18th day, but had not up till then shown trypanosomes. The rat was under observation for two months and trypanosomes were never seen.

RABBIT.—We were unable to utilise rabbits in Rhodesia, but on arrival in Pretoria a rabbit was inoculated intraperitoneally with 5.0 c.cm. citrated blood of Sheep 4. Trypanosomes appeared on the seventh day. Further observations are necessary to determine the constancy of this reaction in rabbits, but there is no reason to consider that any contamination of this strain had been effected during the journey.

DOG 5.—An adult 'Kaffir' dog was inoculated intraperitoneally on October 22nd, 1908, with 10.0 c.cm. citrated blood of Sheep 1. The temperature became irregular from the eighth day and reached 103.6 on the evening of the thirteenth. During the daily examinations to November 15th no trypanosomes were ever seen either in the peripheral blood or on gland puncture, and the dog remained healthy in appearance. On November 27th one trypanosome was seen in a  $\frac{3}{4}$ -inch cover-glass preparation. From that date to January 20th, at Pretoria, they were not again seen.

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Organisms appeared on the sixth day and have been almost constantly present since, though always in small numbers, it being rare to see so many as one to the field, more commonly one to five or ten fields being noted.

The temperature showed little abnormality, and no paroxysmal tendency.

This sheep ('long-eared') was carried to Pretoria where it is under the care of Dr. Theiler who, under date of April 26th, writes that it is still alive.

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No infection resulted.

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August, 1908: White rat 5.0 c.cm. intraperitoneally.

No infection resulted.

FROM CALF.

August, 1908: Guinea-pig 10.0 c.cm. intraperitoneally.

No infection resulted.

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In no case did infection result.

At Kasama, in May, we found a sheep, which had arrived from Lake Bangweolo four days previously, showing trypanosomes which are morphologically identical with those occurring in the Scotsdale

sheep and goat. One inoculation was made, 0.5 c.cm. intraperitoneally into a white rat, without result.

*Morphology of the 'Scotsdale' Trypanosome*

In slides made from the infected animals at Scotsdale, it was seen that the trypanosomes in the sheep and goat differed morphologically from those in the cattle, and the occurrence of a dual infection was suspected. From the observations on Sheep I, however, it was shown that there are variations of the same parasite. We may commence with a description of the forms seen in this animal.

A. '*Long form.*' In fresh preparations the parasite is relatively active, crossing the field with a steady undulating movement of the flagellar extremity, a passage easily followed and one creating but little commotion among the corpuscles. When arrested the movements become more vibratory. On no occasion was the activity manifested by *T. vivax* exhibited.

In stained preparations We employed Giemsa's solution during the earlier part of our work, but later used Leishman's stain freshly prepared in methyl alcohol from the powder made by Grüber. This did not appear to deteriorate as did Giemsa's solution, although this had been taken out in small 10.0 c.cm. bottles.

This trypanosome measured from 20.5 to 25.6  $\mu$  (average 24.74  $\mu$ ) in total length, and 2 to 3  $\mu$  (average 2.7  $\mu$ ) in breadth. A free flagellum was present, accounting for 5.5 to 8.5  $\mu$  (average 6.3  $\mu$ ) of the total. The mean of a series of measurements is as follows:—

Extremity to Blepharoplast	Blepharoplast to Nucleus	Nucleus	Nucleus to body extremity	Free Flagellum
0.85	6.39	3.2	8.0	6.3

The posterior extremity is rounded or bluntly conical; cytoplasm stained a deep pink and is free from granules or vacuoles. Blepharoplast round and prominent, situated about its own length from the extremity. Nucleus compact, but not deeply stained, oval in shape, measuring approximately  $3 \times 1.8 \mu$ , and situated about the centre of the body. The undulating membrane is poorly developed, existing as a narrow band, or, less commonly, showing

one or two small folds. The flagellar rim stains deeply; arising from an achromatic area, it is continued as a free portion of up to  $8.5 \mu$  in length.

B. '*Short forms.*' In fresh preparations this trypanosome is slightly more active than the tadpole of *T. dimorphon*; the difference, however, is only appreciable on close comparison.

Stained, it is indistinguishable from the tadpole forms of *T. dimorphon*. In length it measures from  $10.75$  to  $14.8 \mu$  (average  $13.56 \mu$ ), and is from  $1.25$  to  $1.65 \mu$  in width. The mean of a series of measurements gives:—

Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity
3.94	2.6	7.12

The cytoplasm stains somewhat deeply and is free from granules; vacuoles situated close to the blepharoplast were seen in two or three specimens. The blepharoplast is small and placed close to the extremity; the nucleus, relatively large, stains deeply and measures from  $2.25$  to  $3 \mu$  in length and from  $1$  to  $1.5 \mu$  in breadth. In most individuals it is oval or drawn out, but in some it was almost rounded. There is practically no undulating membrane; and these forms are devoid of any free flagellum.

The original sheep of this strain when examined in May, and from August up to its death, showed only the long forms. This form re-appeared in Sheep 1 on inoculation, and remained present until September 30th. Between that date and October 10th no trypanosomes were seen in this animal, but on their re-appearance they were practically all of the short tadpole variety. A careful examination of some of our films shows that in Sheep 1 prior to September 30th one or two tadpoles were present, and a long form has been seen since October 10th. In the sub-inoculation made on what proved to be the last day of the period of absence, the tadpole form re-appeared in Sheep 4, and is the form which was present at the last examination in Pretoria, January 20th.

The other sheep and the goat at Scotsdale, and the sheep at Kasama, showed the long form; but an occasional tadpole was seen; in the cattle at Scotsdale and Chinsali none but tadpoles were found in the peripheral blood.

In Goat 2 and the Pretoria rabbit, tadpoles appeared; and the one trypanosome seen in a fresh preparation from Dog 5 was also of this variety.

### *Diagnosis*

It may be questioned if these two forms are really of the same species. We think they are. Whether inoculations were made with tadpole or long, the results in white rats and guinea-pigs were invariably negative. Both forms existed at a farm where all animals were herded together and where an autochthonous case is believed to have occurred;\* and examples of both varieties have been seen in films where one or other preponderated. Further, the history of Sheep 1, which we consider was 'clean' at the time of inoculation, indicates a change in morphology rather than a re-infection at our camp, situated nearly 200 miles from the only locality in which a trypanosome non-pathogenic to small animals was seen. That the tadpole was not *T. dimorphon* is, we think, clearly indicated by the failure to infect rats and guinea-pigs with doses of 10 to 30 c.cm. of blood showing trypanosomes.

In morphological characters the 'long' form is almost indistinguishable from *T. vivax* and *T. cazalboui*; it is certainly less actively motile than the Broken Hill *T. vivax*, but no reliance should be placed on such a variable quality. No reference has been made by Ziemann, Laveran or other writer on this species, to the occurrence of any form recalling the tadpole *dimorphon* in naturally infected or sub-inoculated animals; Laveran has had a unique opportunity of observing such forms did they occur in *T. cazalboui*.

The tadpole forms correspond by morphology and animal reaction to the original descriptions of *T. nanum* given by Laveran† and Balfour,‡ and to that of Wenyon,§ who appears to have regained this species in the Anglo-Egyptian Soudan. The last named writer has also described 'long' forms of 'about 20  $\mu$ , 5  $\mu$  of which are taken up by the free flagellum,' and in fig. 39 shows forms which by their rounded posterior extremity, situation of the blepharoplast,

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\* Vide page 333.

† Laveran. *C. R. Soc. Biol.*, 1905, Vol. LVII, pp. 292-294.

‡ Balfour. Second Report of the Wellcome Laboratory, 1906, p. 122.

§ Wenyon. Third Report of the Wellcome Laboratory, 1908, p. 137.



and development of the undulating membrane, recall the 'long' form which we figure in plate. We assume from Wenyon's remarks\* that both 'short' and 'long' occurred simultaneously, but it is to be regretted that a more detailed account of these experiments and observations was not published. The possibility of a mixed infection in a given animal is manifest in a trypanosome-infected continent like Africa, from which already *T. gambiense*, *T. evansi*, *T. brucei*, *T. equiperdum*, *T. dimorphon*, *T. vivax*, *T. nanum*, *T. congolense*, *T. cazalboui*, *T. pecaui*, and *T. sudanense* have been announced in domestic animals. Still greater is the possibility of more than one species occurring in any given herd; one has but to review the recent publications from French West Africa to appreciate this fact, and our own observations at Broken Hill and at Kambole teach the necessity of keeping distinct genealogical records. It is easy to assume the identity of the trypanosomes in the case of two animals infected with such morphologically similar trypanosomes as tadpole *T. dimorphon* (*sensu*, Dutton and Todd, 1903), and tadpole *T. nanum* (*sensu*, Laveran and Balfour, 1905). Rats are inoculated with the latter with negative results; from the former an inoculated sheep is used for all subsequent work, the results of which are directly opposed to those previously obtained, and the inevitable sequel is that considerable confusion occurs in the literature.

The precision and lack of confusion regarding trypanosomes which have been studied in Europe—trypanosomes taken there in individual animals—or in Africa where morphology and diagnosis has been effected by the animal reactions of the strain from one animal alone (e.g., the original *T. nanum* and some trypanosomes of the Sleeping Sickness Commission in Uganda), contrast forcibly with the chaotic state of the literature concerning other African trypanosomes to-day.

*T. nanum* was constituted on the morphological observations of Laveran and the animal reactions noted by Balfour. To be identified with this species, a trypanosome must conform to these original descriptions whatever additional features are brought to light by subsequent investigations.

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\* We have had the privilege of examining some of Dr. Wenyon's specimens. The long form which we describe is morphologically different.

Rigorous rules cannot be laid down for the nomenclature of trypanosomes; but, in general, it may be admitted that *T. nanum* is short and tadpole-like, from 10 to 14  $\mu$ , and is *relatively* innocuous to laboratory animals. A strain virulent to all would cease to be *T. nanum*. It is therefore to be hoped that more details of the strain with which Balfour infected gerbils, and Wenyon infected dogs from which a gerbil was successfully inoculated, will be published. They were derived from different sources, a mule at Wau and a heifer at Sobat; but we do not know whether it is from the *same* animals that the fourteen out of fifteen rats were inoculated without result.\*

We write with the sole idea of analysing the present references to *T. nanum*, and to point out that it is with the original descriptions of this species that we associate the 'Scotsdale' trypanosome, and that we have noted the same morphological variations as has Wenyon. It will, however, be necessary to verify the reaction in the rabbit at Pretoria as being due to the uncontaminated original strain, and to show that it is there still *relatively* innocuous to laboratory animals before accepting that the strain Sheep 4 is that with which the above experiments were carried out.

## 2. Kambole Strains

Kambole is the name of a Mission Station situated on the high land above Lake Tanganyika and the Lovu River, and some fifty miles West of Abercorn. It was selected as suitable locality for an experimental camp owing to the fact that both *Gl. palpalis* and *Gl. morsitans* could be caught within a few miles. One of us arrived there early in August, the other during the third week in September. Until our sudden departure for Europe on November 15th, we had an opportunity of studying the trypanosomes in the mission cattle and of comparing them with the other strains we had accumulated. Only elementary diagnostic work was possible, owing to the great scarcity of small animals: our object was rather to maintain the strains so that they could be brought home, and at the same time arrive at some idea as to the nature of the trypanosomes encountered.

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\* Dr. Wenyon has kindly given us some unpublished particulars of his work on this trypanosome. They indicate forcibly that while rats are insusceptible (he used a wild species), *young* dogs and gerbils may readily take infection. In some cases these animals were inoculated from the same infected beast.

and their 'animal reactions,' that they might be of service in the transmission experiments we contemplated.

The history of the cattle on this station is of interest. Between 1896, when the Mission was established, and 1905, cattle, sheep, goats and pigs are reported to have done exceedingly well. In 1905 the herd of nearly 60 head was divided, half going to the Luapula River, where they have since done well, the others remaining at Kambole. The owner of the latter half was away in May and June of this year, and six weeks after his return (i.e. August) the cattle commenced to die, and twenty were lost before Christmas. In 1906 four deaths are remembered, in 1907 six, and up to our visit in 1908 seven had died. Several additions to the herd had been made by movements from other stations of the same Missionary Society, e.g., Niamkolo and Kawimbi, bringing the number of adults present at the time of examination up to 15. Of these six were infected. The herdman who brought the original stock from the North end of Lake Nyasa in 1896 is still at Kambole, and he is unable to offer any explanation as to why cattle did so well and were free from disease up to 1905, and have since died in such numbers. He avers, and the local natives support him, that these cattle have always grazed on the same areas, and that 'tushembe' was always to be found if the animals wandered far. Unfortunately the name 'tushembe' is applied here to all biting flies, and the natives do not recognise or distinguish *Glossina*, for which this vernacular word is usually restricted. It is possible that an extension of tsetse may have occurred without notice. At the present time *Gl. morsitans* may be taken within three or four miles to the West, and wandering flies were captured on the Mission during our stay near there.

Two of the infected cattle—'Balungu' and 'Ninamwenda'—were selected for the isolation of the strains, since the morphological appearances of their trypanosomes showed some points of difference.

#### A. 'Balungu' strain.

This six-year-old cow was born at Kambole. She showed the hide-bound dejected appearance of a chronic case, with slightly anaemic membranes and enlarged superficial lymphatic glands, but no oedemata. She was examined on five occasions, trypanosomes being present in the peripheral blood on each, and she was destroyed



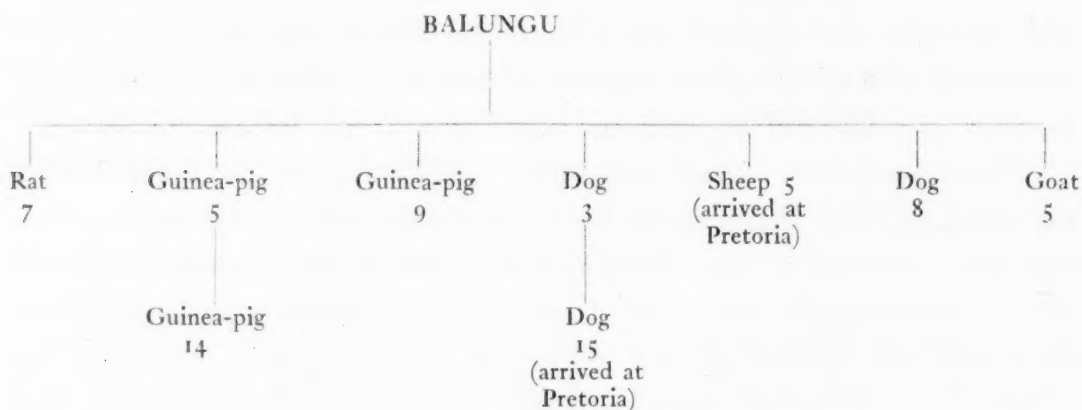
*in extremis* on November 7th, 1908, having been sick for approximately nine months.

*Autopsy.* All subcutaneous tissues were pale and somewhat dropsical. The superficial glands were considerably enlarged and oedematous. The abdominal cavity contained a slight excess of deep citron-coloured fluid. The peritoneal covering of the rumen, reticulum, spleen and areas of the intestine was studded with petechiae, in parts congregated into patches two inches in diameter. The mesentery was infiltrated with a gelatinous material. The liver was slightly congested; the gall-bladder distended and contained over 900 c.cm. of reddish watery bile. The spleen was somewhat enlarged, the surface studded with petechiae, the pulp dark and friable, and Malpighian bodies prominent.

There was no excess of fluid in the thoracic cavity or pericardial sac. Slight lobar congestion and emphysema of both lungs. The heart, pale and very flabby, showed fatty degeneration; the base was surrounded by a quantity of gelatinous material replacing all fat.

The mesenteric and mediastinal lymphatic glands were enlarged and oedematous and some of them were congested.

The following is the genealogical record of this strain:—



### *Experimental infection*

RAT 7.—Considering it possible we might be dealing with 'Scotsdale' Strain, 10 c.cm. blood of naturally infected cow was inoculated intraperitoneally into this animal, on October 23rd. Negative on the 29th; trypanosomes were seen on November 1st and remained present until death on November 10th—17 days.

GUINEA-PIG 5.—October 8th, 1908. Inoculated intraperitoneally with 5 c.cm. blood of naturally infected cow. Almost daily examination up to November 10th failed to reveal any infection, so the animal was re-inoculated on this date with about 2.0 c.cm. heart blood of Rat 7 just dead. Trypanosomes were seen on November 23rd, the first examination since November 13th, and were present at succeeding observations till death on December 12th.

GUINEA-PIG 9.—Owing to the initial failure to obtain infection in Guinea-pig 5, this animal received 10.0 c.cm. intraperitoneally on October 23rd. Trypanosomes first seen November 1st; they were not seen on the 5th or 7th, but thereafter were constantly present—but *always in small numbers*, 2 in  $\frac{1}{4}$  of a cover ( $\frac{3}{4}$  inch) being the maximum noted until a week prior to death, which took place on December 4th (41 days), when they became more numerous, up to two per field.



GUINEA-PIG 14.—December 12th. Inoculated intraperitoneally with 0.5 c.cm. heart blood of Guinea-pig 5. Trypanosomes were seen one to a field on December 18th, and the animal died on December 22nd. Death was undoubtedly largely accelerated by, if not due to a vegetable poisoning which accounted for the loss of three other guinea-pigs the following day.

DOG 3.—October 8th, 1908. Inoculated intraperitoneally with 5.0 c.cm. blood of Balungu. The temperature rose to  $102^{\circ}$  on the fourth day, and continued slightly elevated between  $102^{\circ}$  and  $103^{\circ}$ , with few exceptions, to death. Trypanosomes appeared 1 in  $\frac{1}{4}$  cover on the eighth day, and remained present in small numbers, 1 in 10 fields being the maximum, up to November 15th when they were about 12 to a field. Death took place on November 21st—44th day after inoculation.

DOG 8.—October 31st. Inoculated subcutaneously with 1.0 c.cm. blood of Balungu. Temperature rose almost immediately to  $102^{\circ}$  and continued irregular so long as it was taken. Trypanosomes were never seen in the blood, but gland puncture on the 10th and 19th day after inoculation showed them to be present. Death took place on December 4th (35 days) from pneumonia. Trypanosomes could not be demonstrated, and the spleen was normal.

DOG 15.—November 25th. Inoculated subcutaneously with 3.0 c.cm. heart blood of Dog 3 just dead. Trypanosomes were never seen in the blood or gland juice of this animal up to his arrival in Pretoria.

SHEEP 5.—October 23rd, 1908. Inoculated intraperitoneally with 6.0 c.cm. blood of Balungu. Trypanosomes were seen 1 in  $\frac{1}{4}$  cover on the sixth day, and were subsequently found in small numbers (1 in 1 on one occasion only). The temperature showing indications of the Broken Hill *dimorphon* type, but less marked. This animal was taken to Pretoria, and was still living on January 23rd. Dr. Theiler informs us on April 26th, 1909, that this sheep has since died.

GOAT 5.—October 31st, 1908. Inoculated subcutaneously with 1.0 c.cm. blood of Balungu. The temperature rose steadily and reached  $106.4^{\circ}$  on the evening of the eighth day, and continued irregular. Trypanosomes were not seen in the peripheral blood until the 14th day (1 in  $\frac{1}{4}$  cover); they had, however, been seen in the glands since the ninth day. The animal was destroyed when the infection was realised.

#### *Morphology of 'Balungu' trypanosome.*

The trypanosomes seen in the original cow and the sub-inoculated animals, rat, guinea-pigs, dogs, sheep and goat, are of the same appearance. They correspond closely with the tadpole form of *T. dimorphon* (Plate IV, fig. 3).

In fresh preparations the movements are localised to the field, and are not very active.

In stained preparations the length varies from 10.2 to 16.3  $\mu$  (average 13  $\mu$ ), the larger being divisional form, and the breadth from 1.0 to 2.2  $\mu$  (average 1.5). The following is the mean of a series of measurements:—

Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity
4.93	2.07	6.08

The blepharoplast is not quite terminal (0.5 to 1.0  $\mu$  from the end); the nucleus is oval or compressed, and is placed rather towards the posterior extremity. The undulating membrane is rudimentary, and there is no appreciable free flagellum. The posterior extremity is rounded or bluntly conical. The cytoplasm is commonly homogeneous, but in some slides a considerable proportion show a clear area (? vacuole) near the blepharoplast, whilst many exhibit a few fine chromatic granules between the blepharoplast and the nucleus. The greatest width is posterior to the nucleus.

#### Diagnosis

From its morphology it is quite evident that this trypanosome is not related to the *evansi* group; and by its pathogenicity to small animals it is separable from the *vivax* group, and consequently from *T. nanum*, though on morphological grounds alone it would be impossible to distinguish it from the tadpole forms of this latter.

According to our rough grouping\* it would fall under the head of *dimorphon*, here containing the three named forms *T. dimorphon*, *T. congolense*, and *T. pecaui*. Owing to the entire absence of long free-flagellated forms, and the accompanying broad individuals with well-developed undulating membrane, the last named may be excluded. We have then to discuss the properties of *T. dimorphon* and *T. congolense*, the only remaining named species, and to ascertain to which the organism from Balungu most closely approximates. It is to be noted that the 'animal reactions' in all these are closely similar.

According to the original descriptions of Dutton and Todd,† *T. dimorphon* occurs in at least two forms:—(1) a tadpole similar to that which we have just described, and (2) a long form carrying a free flagellum. Intermediate forms, termed stumpy, may also be found.

This parasite was found in the Gambia, where ten horses were detected as suffering from trypanosomiasis. Of these, four were used for experimental observation—Cases 1, 5, 6 and 9. Case 6 was brought to England and served for the experimental work carried out by Thomas and Breinl‡ and by Laveran and Mesnil§. Practically all

\* Montgomery R. E. and Kinghorn, A. *Ann. Trop. Med. Parasitol.*, 1909, II, 5, pp. 333-344.

† Dutton and Todd. *Thompson Yates and Johnston Lab. Reports*, 1903, Vol. V.

‡ Thomas and Breinl. *Thompson Yates and Johnston Lab. Reports*, 1905, Vol. VI, II.

§ Laveran and Mesnil. *Trypanosomes et Trypanosomiasis*.

Dutton's and Todd's work was conducted on Case 1, the strain from which was not used in Europe, and it is from this animal that the microphotographs (Plate IV, figs. 2 and 3 of their Report), which clearly show the 'stumpy' and the free-flagellated 'long' forms, were made. We elsewhere analyse the published evidence on this question; here it is sufficient to say that we consider it highly probable that Horses 1 and 6 were infected with distinct trypanosomes, one of which (Horse 1) was the original host of *T. dimorphon*. The other (Horse 6) is the original host of that parasite, which we have designated *T. confusum*, which at Liverpool and Paris has been accepted as *T. dimorphon* that had undergone a peculiar morphological transformation. We hold that we have recovered *T. dimorphon* at Broken Hill, thereby reducing the strength of an argument that Dutton and Todd were concerned with an infection by such a trypanosome as that now known as *T. pecaui*, or were misled by a mixed infection. We consider that our work at Broken Hill may be considered to substantiate the species in conformity to the original description.

For a trypanosome to be regarded as *T. dimorphon* it is necessary that it be shown to develop a free flagellum. From the observations of Dutton and Todd in the Gambia, and ourselves in Northern Rhodesia, it would appear that small experimental animals are best suited for this purpose. 'Long' forms were produced in all five of our guinea-pigs, and in three out of five white rats inoculated with this strain of trypanosome in its first, second and third passages.

In one rat, three guinea-pigs and two dogs infected by the 'Balungu' strain, we failed to notice the development of a free flagellum, or even the occurrence of forms corresponding to the 'long form' of Laveran and Mesnil. The longest specimen we measured was  $16.3 \mu$ . The evidence is negative, but with such as it is we cannot identify this trypanosome with *T. dimorphon*. It then falls into the group represented by *T. confusum* (*T. dimorphon* of Paris), and *T. congolense*. Laveran\* has drawn attention to the differences between these species. He writes:—'Au point de vue morphologique, *Tr. congolense* diffère de *Tr. dimorphon*. Le premier de ces trypanosomes mesure  $10 \mu$  à  $13 \mu$  de long, les exemplaires qui

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\* Laveran. *Annales de l'Institut. Past.*, 1908.



'atteignent  $15\ \mu$  à  $17\ \mu$  de long sont fort rares; *Tr. dimorphon* présente au contraire, dans les cas types, un mélange de petites formes ( $10\ \mu$  à  $15\ \mu$  de long) et de grandes ( $22\ \mu$  de long en moyenne). . . . Mais *Tr. dimorphon* ne se présente pas toujours sous ses formes typiques. Dans certaines infections dues à *Tr. dimorphon*, les grandes formes sont rares ou très rares; si bien qu'on pouvait supposer que *Tr. congolense* était une variété de *Tr. dimorphon* dans laquelle les grandes formes avaient disparu. . . .', and as further substantiating Broden's species he quotes the results of 'cross inoculations' by *T. dimorphon* (*T. confusum*) into animals 'immunised' towards *T. congolense*. These observations on the morphology are in entire accord with those of Broden,\* who gives a maximum measurement of  $15.5\ \mu$  for his species.

The name *Trypanosoma congolense* cannot then be applied to a form which under the normal conditions of experimental observation exceeds about  $17\ \mu$  in length; it differs therefore from *T. confusum*, which may attain  $23$  or  $24\ \mu$  but which does not develop a free flagellum, and from *T. dimorphon*, which in certain forms resembles these two exactly, but which is capable of developing a distinct free flagellum upwards of  $10\ \mu$  in length. The parasite which Höhnelt has described under the name *T. congolense* would appear to approximate more closely with *T. confusum*.

The trypanosome from Balungu has not been seen to exceed  $16.3\ \mu$ ; until further investigation decides that it is capable of assuming larger dimensions, in which case our conception of the classification of this group must be amended, we associate it with *T. congolense*, *sensu* Broden and Laveran.

#### B. *Ninamwenda* strain.

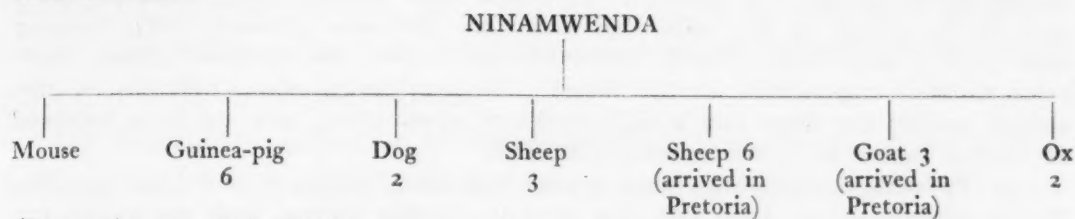
This cow came to Kambole in August, 1907, from the Niamkolo Mission on Lake Tanganyika, a place where cattle appear to thrive and where no sickness is recorded. In the rainy season of 1907-1908 she became extremely emaciated and lost practically all her hair, but regained condition and coat in the spring of 1908. At examination on October 1st, she was not particularly thin, and was said to be improving; trypanosomes, one in fifteen fields, were seen, and they

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\* Broden. *Rap. Lab. Leopoldville*, No. II, 1906.



were present at each succeeding examination till our departure. The owner and the herdsman considered her to have recovered her original good health, and said she gave a normal amount of milk and nourished her calf well. The following inoculations were made from this animal:—



### *Experimental infection*

GREY MOUSE caught locally. Inoculated October 23rd with 0.4 c.cm. direct from Ninamwenda. No trypanosomes were seen in its blood up to November 6th, when it escaped.

No RATS were available for inoculation.

GUINEA-PIG.—October 8th, 1908. Inoculated intraperitoneally with 10.0 c.cm. blood of Ninamwenda. Trypanosomes were not seen at almost daily examinations until November 3rd—the 26th day. They disappeared for five days then returned, being present at each subsequent examination in numbers varying from 1 in  $\frac{1}{4}$  cover-glass to 1 in a field—an average would be one to eight or ten fields. The animal maintained its condition well, despite the rough usage and exposure on the march, and was accidentally killed on January 3rd, 1909. There were no abnormal lesions on autopsy, and the body was well nourished and fat.

DOG 2.—October 8th, 1908. Inoculated intraperitoneally with 10.0 c.cm. blood of Ninamwenda. From the 8th day the temperature became irregular and jerky, and the dog lost condition, acquired a harsh coat and appeared depressed. A month after inoculation there was considerable oedema of the sub-maxillary space and a catarrhal discharge from the eyes. He died in an emaciated state on November 29th (52 days), without ever showing trypanosomes in his blood or on gland puncture. Post-mortem showed only general enlargement of all lymphatic glands, and considerable oedema in the connective tissues of throat and in the body cavities. The spleen appeared normal. No sub-inoculations were possible, but a careful search failed to show trypanosomes in any organ.

SHEEP 3.—An aged male. October 8th, 1908. Inoculated subcutaneously with 1.0 c.cm. blood of Ninamwenda. Trypanosomes first appeared on the 23rd day at which time the temperature rose slightly. They were then present daily, with two exceptions, till the animal was destroyed on our leaving for home. They were never numerous—1 to two fields being highest recorded, and the temperature was but slightly irregular, 104.8° maximum.

SHEEP 6.—October 23rd, 1908. Inoculated intraperitoneally with 3.0 c.cm. blood of Ninamwenda. Trypanosomes, 1 to  $\frac{1}{4}$  cover-glass, were seen in the 11th day; on the 12th, 13th, 14th they were absent, but reappeared on the 15th, from which day they were always present in small numbers—1 to 10 fields maximum. The temperature showed very little irregularity, and the animal travelled well, reaching Pretoria in January in good condition, and according to Dr. Theiler's letter of April 26th, was then still alive.

GOAT 3.—About three months, still with its mother. October 23rd, 1908. Inoculated intraperitoneally with 3.0 c.cm. blood of Ninamwenda. One trypanosome was seen on the 19th day, but not again during the regular examinations. On the march they were seen twice in five observations and were

present on this animal's arrival in Pretoria. There was no apparent loss in condition and no symptoms were manifested, and this animal was alive on April 26th, 1909.

Ox 2.—1½ years old. October 23rd, 1908. Inoculated subcutaneously with 5.0 c.cm. blood of Ninamwenda. The temperature rose on the eighth day to 103° and continued in that neighbourhood to the 17th, when it rose to 105.2°, falling again on the 25th when temperatures were ceased. Trypanosomes were seen in the blood on the 10th day, and were thereafter present daily, ranging from 1 to 3 to a field. Gland puncture had shown the infection three days prior to their appearance in the blood. No symptoms were noticed in the animal during the three and a half weeks of observation, and we have received no further report as to his present condition.

At Pretoria, inoculations were made from both Sheep 6 and Goat 3. Dr. Theiler informs us on April 26th that from the former animal both the guinea-pig and the rabbit became infected, from the latter the guinea-pig is positive, but the rabbit is negative.

#### *Morphology of the 'Ninamwenda' Trypanosome*

In fresh preparations the trypanosomes from this cow and also from a second (Nakakoti) were clearly distinct from those of the *T. congolense* type seen in Balungu. They appeared as slightly elongated, egg-shaped bodies, moving across the field with the flagellar extremity in front, and rarely showing any movement of the body, even when held up by corpuscles. They easily traversed the field of vision, but at a rate at which they were readily followed.

Stained by Leishman's method, they measured from 11 to 19  $\mu$  in length, the latter representing divisional forms (average 14.66  $\mu$ ), and from 2.9 to 3.75  $\mu$  in breadth (average 3.38  $\mu$ ). The mean of a series of measurements is:—

	Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity	Portion of free flagellum
	5.45	2.36	5.1	1.7
(In Nakakoti)	4.97	2.26	4.89	1.48

In Nakakoti the average width was 3.58.

The blepharoplast is almost terminal or up to 0.8  $\mu$  from the end. The rounded or oval nucleus is almost centrally placed. The undulating membrane is represented by a narrow band running parallel to the body, being better developed in the narrower forms. The rather well-defined rim is continued as a bristle-like projection of up to 1.7  $\mu$  in length, hardly amounting to a flagellum, but free of ectoplasm. The posterior extremity is rounded or very bluntly angular, the flagellar abruptly drawn out. The cytoplasm stains a deep pink, and commonly shows a large and most distinct vacuole just posterior to the nucleus. Though not always present, or not so prominent, this

was of very frequent occurrence, as also was the existence of chromatic granules at both poles of the nucleus.

### Diagnosis

The picture presented by the trypanosomes is striking, and dissimilar to all other forms; the ratio of breadth to length (1 : 4), the round aflagellar extremity and the narrow undulating membrane giving it a characteristic shape.

On careful examination of some slides (Ninamwenda) a few forms are seen which individually would be considered as not unlike a broad tadpole of *T. nanum* or *T. congolense*, but they are most exceptional (the smallest is seen on Plate IV, fig. 1). In the successful sub-inoculations at Kambole (guinea-pig, sheep, goat, and ox) only the characteristic broad forms were seen. Sheep 6 and Goat 3 were taken to Pretoria, where it was found that the latter was still showing this type. The trypanosomes in Sheep 6 were identical morphologically with the tadpoles of *T. nanum* or *T. congolense*. We are quite unable to explain this at present. The change may be due to a natural alteration in the morphology, or to a secondary infection acquired on the march down. During the journey of six weeks from Tanganyika to the railway these animals were carried in large fly-proof cages, so that *Glossina* may be absolutely excluded. In country known to be free from tsetse they were allowed out for a short time on arrival in camp at night, and it might be considered possible for infection to have then occurred; or, as three sheep or goats were taken in each cage, it may have resulted without liberation. It is hoped that Goat 3 may serve as a control to this change: with both animals in Pretoria, an exact and exhaustive comparison may be made. Until this has been accomplished we hesitate to do more than assign this trypanosome to the group containing *T. dimorphon*, and note that in the original and all sub-inoculated animals at Kambole it presented morphological features unusual in the other members of the group, features which if maintained in successive passages would warrant a conclusion that this trypanosome differs from previously described species.\*

\* I have been authorised by Dr. Laveran to say that he has examined the preparations of Mr. Montgomery and Dr. Kinghorn, labelled '*Ninawenda strain*,' coming from a Rhodesian cow.

Dr. Laveran adds, 'Le trypanosome est voisin de *Tr. congolense* mais beaucoup plus large que ce dernier, ce qui permet de croire qu'il s'agit (encore!) d'un espece nouvelle.' He proposes if the trypanosome is really a new species, to call it *Tr. montgomeryi*, and I may add that Dr. Kinghorn agrees to this proposal.—R. Ross.



# TRYPANOSOMES OCCURRING IN NATURALLY INFECTED DOGS

## A. *The 'Chunga' Trypanosome.*

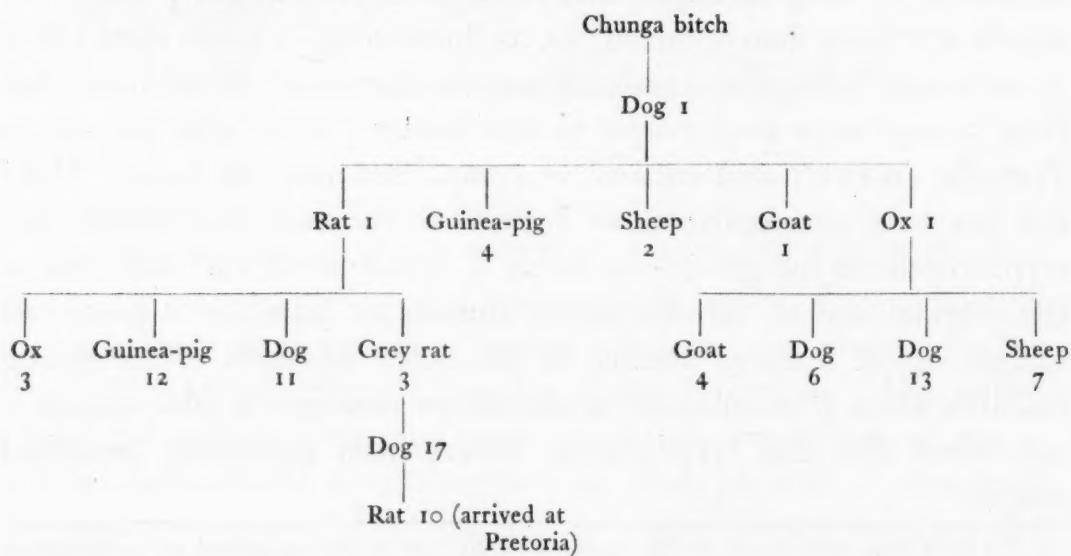
Origin. This strain was derived from an Airedale bitch which was brought North from Cape Colony at the end of May. It left the railway on July 7th. On the 5th August the owner, who was travelling with her, noticed she was getting rather weak, and that her milk was diminishing—this was ascribed to the litter of pups which had been born on July 18th—but she appeared to regain her condition. On August 27th an opacity of the right cornea was noticed, and examination on September 4th at Chunga Farm showed trypanosomes.

Tsetse flies (*Gl. morsitans*) might have been encountered within a few days of the railway. They appear to be constantly present in a district (Mpika) entered on August 1st; and it would seem possible for infection to have taken place then.

We were informed that this bitch was destroyed *in extremis* on September 11th, a week after diagnosis.

A healthy native dog was inoculated from this bitch and was carried to Kambole, serving as the origin for the experimental work possible; it was, however, rather our desire to maintain the strain for future investigation under suitable conditions.

The following is the genealogy of the strain:—



## *Experimental infection*

RAT 1.—This animal had been inoculated on May 9th with 'Scotsdale' strain without result.



September 24th. Received intraperitoneally three drops blood of Dog 1. Trypanosomes appeared on the fifth day and were constantly present till death on November 9th (46th day). Ten days before death there were incoördination of the hind legs, and a muco-purulent discharge from the conjunctivae.

RAT 10.—Was inoculated subcutaneously with 0.5 c.cm. heart blood of Dog 17 just dead, on December 31st. No further examinations were made till the animal arrived in Pretoria, when organisms were very numerous. It was still alive on January 23rd.

GREY RAT. 3.—Caught locally. Inoculated subcutaneously November 9th, 1908, with 0.5 c.cm. blood of Rat 1. Trypanosomes appeared on the fourth day and were constantly seen up to death on December 14th, the 35th day.

GUINEA-PIG 12.—This animal had been unsuccessfully inoculated with 'Scotsdale' strain in May, 1908.

November 3rd, inoculated with eight drops blood Rat 1, and on this rat's death, November 9th, received the heart washings. Trypanosomes were seen on November 13th, ten days after first inoculation. They were present later at various times up to death, which took place on December 23rd, and was probably due to a poisonous grass in the food (vide Scotsdale guinea-pig). Trypanosomes were not seen on autopsy, and the body was well nourished.

GUINEA-PIG 4. An animal which had resisted infection by *T. vivax* a year previous.

September 24th, 1908. Inoculated intraperitoneally with three drops of blood from Dog 1. The animal remained in perfect health, and trypanosomes were never seen at frequent (every two days) examinations up to November 5th. Organisms were first seen on November 5th, and were thence present at most subsequent examinations till death on December 17th, which was without doubt accelerated by frequent exposure to the rains and cold weather and by constant travelling.

DOG 1.—A young native bitch. Inoculated subcutaneously September 5th, 1908, with twelve drops blood of original case. Organisms were not seen on September 12th, the only examination prior to arrival at camp, September 22nd. From this date to that of death, October 18th (thirty-three days), trypanosomes were constantly present. Beyond emaciation and weakness no special symptoms were observable—there was no corneal opacity as in the original animal. The temperature was constantly elevated to 102° to 103°, and showed no paroxysmal tendency.

DOG 6.—October 30th, 1908. Received 6 c.c. subcutaneously of this strain passed through Ox 1, trypanosomes 1-4 fields. It did not become infected, and died of pleuro-pneumonia on December 21st.

DOG 11.—November 1st, 1908. Inoculated subcutaneously with twenty drops blood from Rat 1. Temperature commenced to rise on the fifth day, and trypanosomes one to a field were seen on the sixth. During the time it was taken, the temperature was considerably elevated, reaching 105.4°, and trypanosomes were constantly present till death on December 10th (forty days).

DOG 13.—This dog served as a control to Dog 6, which did not become infected after inoculation from Ox 1. November 9th, 1908. Inoculated intraperitoneally with 30 c.c. from Ox 1 (trypanosomes 1-30 fields). The temperature commenced to rise on the fifth day, and trypanosomes were seen on the seventh and at succeeding examinations up to death on November 27th—18th day.

DOG 17.—December 14th. Inoculated subcutaneously with heart blood of Grey Rat 3. Trypanosomes were very numerous at the first examination on the eighth day. The disease was acute and the dog died on December 31st—16th day.

**SHEEP 2.**—September 29th. Inoculated subcutaneously with three drops blood of Dog 1. Trypanosomes one to a cover were seen on the eleventh and twelfth day, when the animal was destroyed.

**SHEEP 7.**—November 14th. Inoculated intraperitoneally with 10.0 c.c. blood from Ox 1. The object of this experiment was to note whether the morphological forms present in this Ox would reappear. Trypanosomes 1 in  $\frac{1}{4}$  cover-glass were seen in the blood on one occasion only (December 15th): they were present in the glands on December 1st, 10th and 23rd, and not seen on December 14th. This animal died in the train on January 8th, and was too decomposed for inoculation on arrival in Pretoria the following day.

**GOAT 1.**—An adult female. September 30th. Inoculated subcutaneously with three drops blood from Dog 1. The temperature rose on the sixth day and again on the tenth ( $106.8^{\circ}$ ), from which date it was almost continually elevated above  $104^{\circ}$ , and after the fifth week was over  $105^{\circ}$ . Trypanosomes were only seen twice on direct examination of the blood—1 to half a cover-glass ( $\frac{3}{4}$ -inch) on each occasion—the 18th and 27th day. They could constantly be found on puncture of the prescapular glands. No symptoms were noted beyond slight emaciation, the animal retaining its strength until it was destroyed on our leaving for home.

**GOAT 4.**—Adult female. October 30th was inoculated subcutaneously with 4.0 c.c. blood from Ox 1. The temperature commenced to rise on the fourth day and continued irregular, but always elevated between  $103^{\circ}$  and  $107^{\circ}$ , till it was destroyed on November 15th. Trypanosomes were never seen in the peripheral blood; but they were constantly present in the prescapular gland from the seventh day.

**Ox 1.**—A five-year-old cow. Had been under observation for eighteen days prior to inoculation, during which she was in perfect health and her temperature had never exceeded  $102^{\circ}$ .

October 18th, was inoculated subcutaneously with 8.0 c.c. from Dog 1, just dead. The temperature commenced to rise the following day ( $103.2^{\circ}$ ). On the third day it was  $105^{\circ}$ , and  $106^{\circ}$  on the fourth after inoculation, and then fell to  $103.2^{\circ}$ . For a week it continued irregular, showing a daily variation of two to three degrees, later becoming more even, but ranging daily from  $101.5^{\circ}$  to over  $103^{\circ}$ . Trypanosomes were first seen 1 to half a cover-glass on October 24th—the sixth day—and they were thereafter continually present, averaging one to ten fields: on two occasions only did they exceed one to a field. There was therefore no periodicity in either temperature or organisms; and no increase in numbers when a high temperature ( $106.2^{\circ}$ ) was registered early in the infection. Gland puncture showed trypanosomes two days prior to their appearance in blood. During the four weeks of disease she was under observation, there was noticeable emaciation, the animal being in very good condition at the time of inoculation. At our departure she was left under the charge of a neighbouring European; but up to date we have no report as to her condition.

A suspicion was aroused that by some means a mixed infection might have occurred, and a series of inoculations was carried out to ascertain this point. We were, however, unable to obtain any evidence of such an occurrence.

**Ox 3.**— $1\frac{1}{2}$  years old. This Ox was inoculated to see whether the trypanosomes noted in Ox 1, which were suspected as being foreign to the inoculation, would again reappear.

October 31st. Inoculated subcutaneously with 20 drops blood from Rat 1. There was a slight thermal reaction sixth and seventh days—from then to the fifteenth, the day we left, it continued normal. No trypanosomes were seen in the peripheral blood during this period, but gland puncture was positive from the seventh day. We have no report as to the present condition of this Ox.

*Morphology of 'Chunga' Trypanosome**(a) In original case*

In fresh preparations the trypanosomes were long and caused considerable disturbance among the corpuscles; they remained localised to the field.

Stained, two main types were discernible.

1. A 'long' form measuring from 24.75 to 30.3  $\mu$  in length (average 26.9  $\mu$ ) by 1.5 to 2.0  $\mu$  in width. An average measurement of a series is:—

Extremity to Blepharoplast	Blepharoplast to Nucleus	Nucleus	Nucleus to extremity	Free Flagellum
3.25	5.62	3.75	5.62	8.74

The body is narrow, the posterior extremity drawn out into what Balfour describes as a 'pike's head'; the other is continued as an ectoplasmic prolongation for a variable distance along the flagellum; the cytoplasm stains faint bluish and is relatively free from granules or vacuoles. The blepharoplast is removed about 3.0  $\mu$  from the extremity, and the elongate nucleus (3.75  $\times$  1.5) lies well to the flagellar end of the body. The undulating membrane is fairly well developed, though hardly so much so as is usual in *T. evansi*; the rim is continued as a free flagellum varying from 6 to 11  $\mu$  in length, clear of all ectoplasm.

2. 'Short' forms measuring from 17.15 to 21.9  $\mu$  in length (average 19.0  $\mu$ ) by 2 to 3.5  $\mu$  (average 2.6  $\mu$ ) devoid of a free flagellum, or having only a beak of one or two micra. The following is the mean of a series of measurements:—

Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity
6.55	2.2	10.25

The body is relatively broad; the posterior extremity is bluntly angular or sometimes rounded; in a few cases it showed evidence of being drawn out. The cytoplasm stains more pink than is seen in the 'long' forms, and granules, though rare, are of more common occurrence; vacuoles were not noted. The blepharoplast is removed from 0.7 to 1.5  $\mu$  from the extremity or may be almost terminal.



The nucleus is round and lies slightly on the aflagellar side of the middle. The undulating membrane exists, but is not well developed, and the rim ceases with the body, or may be continued in a few instances for as much as  $4\ \mu$ .

In this dog, the long forms greatly exceeded in number the short, at the time of examination. Intermediate forms were very uncommon.

(b) *In experimental animals.*

(i) In the *rats*, *guinea-pigs* and *dogs* both forms were seen but the short was usually in numerical excess of the large; this latter was not seen in certain slides from Dog 11, and in some others it was very rare. In Rat 1 they co-existed in almost equal numbers.

(ii) The extreme rarity of occurrence in the blood of *sheep* and *goats* precluded a study of the forms; in smears made from gland punctures they respond to the 'short' forms, but the length is greater (average  $23\cdot2$ ); the free flagellum was rudimentary.

(iii) In *cattle*. In fresh preparations the motility was found to vary from day to day, and also in individual trypanosomes. Most commonly the form which re-appeared was almost as active as that to which we have referred as *T. vivax*; less frequent were those trypanosomes whose gliding movements without corpuscular displacement could be watched while crossing a field. Only rare were those even temporarily remaining in a single field.

In stained preparations we have only seen one form. This trypanosome showed morphological variations from those in other animals inoculated with this strain. It measured from  $21\cdot75$  to  $25\cdot5\ \mu$  in length (average  $22\cdot4\ \mu$ ) and from  $2\cdot7$  to  $4\cdot5\ \mu$  in breadth (average  $3\cdot3\ \mu$ ). The following is the mean of made measurements:—

Extremity to Blepharoplast	Blepharoplast to Nucleus	Nucleus	Nucleus to body extremity	Free Flagellum
0·7	5·8	3·3	7·3	5·52

The body is rounded or bluntly conical at the posterior extremity, and tapers gradually from the widest point, which is close to the region of the blepharoplast. The cytoplasm is commonly homogeneous and free from chromatic granules or vacuoles. The blepharoplast is



large and  $1.0\ \mu$  from the end, but frequently it is almost terminal; the nucleus is compact, round or somewhat oval, and situated towards the middle of the body. The undulating membrane is represented, as in *T. vivax* and most of the long forms of *T. nanum*, by a narrow band extending parallel to the margin of the body; its rim is continued as a free flagellum of from  $4.0$  to  $6.0\ \mu$ .

In its morphology this trypanosome so closely resembled that which we described as *T. vivax* at Broken Hill that we suspected a mixed infection—a suggestion accentuated by the rapid rise in temperature after inoculation, and by the failure to infect Dog 6. *T. vivax* was not rare in the blood of inoculated sheep and goats, while, as indicated by Sheep 2 and Goat 1, the Chunga organism was uncommonly seen. Goat 4, Sheep 7 and Dog 13 were inoculated from this cow; the two ruminants became infected without showing numerous trypanosomes in the blood (observation on Goat 4 limited to fifteen days; no peripheral organisms seen, though gland puncture positive); the dog became infected with trypanosomes identical to those seen in the original and sub-inoculated dogs, the short forms markedly preponderating. As a control to these experiments a second ox was inoculated with this strain passed through a rat. Unfortunately we had to leave for home before this animal showed peripheral trypanosomes; but a comparison of forms seen in gland-puncture specimens from these two bovines did not reveal any appreciable differences. We are therefore led to consider it possible that the Chunga strain passed into a bovine may manifest this morphological variation, and that the failure to infect Dog 6 was due to individuality.

### Diagnosis

Owing to lack of comparative study we are unable to assign this trypanosome to any particular species. In our original dog and in several of the sub-inoculations it shows considerable affinity in both morphology and animal reaction with the *T. evansi* group, and we have spoken of it as allied to *T. brucei*. In the strain of *T. brucei* maintained at Runcorn, forms corresponding to both 'long' and 'short' are found—the so-called 'male' and 'female'—but on an average they are larger, and more transitional stages can be made out. It will at once be remarked by one conversant with Laveran's

work\* that the description we have given of the 'Chunga' trypanosome shows a marked similarity to *T. pecaudi*. It is to be observed, however, that we have never seen a trypanosome of  $14.0\ \mu$ ; our smallest was  $17.15\ \mu$ ; the undulating membrane in our form is less developed than that figured by Laveran, and we have the more common occurrence of a small flagellum. This trypanosome is maintained at Pretoria, and additional work will make its position clearer.

#### B. *The 'Wallace' trypanosome*

In September we examined an Irish terrier which had accompanied the Administrator, Mr. Wallace, on tour from Fort Jameson. Trypanosomes were present in his blood, but the dog died and was destroyed before we got an inoculation. Mr. Wallace thereupon very kindly telegraphed to Fort Jameson for two dogs to be sent in charge of messengers to follow the same route. These dogs, born in Fort Jameson, and apparently in perfect health, left there on September 24th and travelled *via* Nawalia, Mpika, Kasama and Abercorn. One reached our Kambole camp on October 29th; the other is said by the messengers to have died of extreme weakness two days before.

This dog 'Dip,' a short-haired animal of about 35 lbs. weight, was in hard condition on arrival from the long march; on casual glance he was quite healthy and strong, only his membranes were somewhat pale. Trypanosomes were present in his blood.

The dog refused food on November 1st, and on the 2nd was unable to stand and was semi-comatose; his coat was staring and respirations increased. During the morning he had five epileptiform fits, lasting one-half to three-quarters of a minute, during which he made violent attempts to rise, and snapped at any near object, howling and attempting to bark whilst the fit lasted. As no improvement was noticed, he was destroyed by an intrapleural injection of hydrocyanic acid at four o'clock. During these few days his temperature had not exceeded  $102.5^{\circ}$ .

*Autopsy.* Membranes and subcutaneous tissues somewhat pale, but not excessively so. There was no increase of fluid in the body cavities. Lymphatic glands were all enlarged, slightly oedematous and congested. Other organs

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\* Laveran. *Annales de l'Institut. Past.*, 1907.

healthy-looking, except the spleen, which was of enormous size: it extended right across the abdomen and measured over three inches in width at the narrowest part, and was thickened to an extent of nearly two inches; the substance was soft and friable, and the Malpighian bodies large and prominent.

### *Experimental infection*

The following inoculations were carried out:—

RAT 8.—October 30th, 1908. Inoculated subcutaneously with 2.0 c.c. blood of 'Dip.' Trypanosomes appeared on the fifth day and increased in numbers to death on November 10th.

GUINEA-PIG 10.—October 30th, 1908. Inoculated intraperitoneally with 7.0 c.c. blood of 'Dip.' No trypanosomes were seen up to November 15th. They were present on November 22nd and at succeeding examinations to death, which occurred on December 7th (38th day).

GUINEA-PIG 13. December 7th. Inoculated intraperitoneally with 1.5 c.c. blood direct from Guinea-pig 10. Trypanosomes were seen on December 12th and the animal died on December 14th, a date on which several of our guinea-pigs died, as a result, we think, of some vegetable irritant in the food.

DOG 12.—November 2nd. Inoculated subcutaneously with 5.0 c.c. heart blood direct from 'Dip' on post-mortem. The temperature rose on the sixth day, and trypanosomes were present. They increased in numbers, and the temperature continued to rise up to death on November 13th, the eleventh day.

DOG 14.—Was inoculated subcutaneously with 3.0 c.c. blood of Dog 12 on November 13th. No trypanosomes were seen up to December 7th, when he was stolen or lost.

DOG 15.—Was inoculated subcutaneously on December 14th with 2.0 c.c. heart blood of Guinea-pig 13 immediately after death. No trypanosomes were ever seen in the blood of this animal, which arrived in Pretoria looking well.

### *Morphology of the 'Wallace' Trypanosome*

In fresh preparations the actively moving organisms remain localised to the field and rarely attempt to travel; they recall the forms seen in Balungu (Plate IV, fig. 4).

Stained, they present the ordinary appearance of a 'tadpole' trypanosome, measuring from 8.8 to 15.3  $\mu$  (average 12.3  $\mu$ ) in length, and from 0.9 to 1.75  $\mu$  in width. The following is the mean of the measurements:—

Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity
4.4	2.1	5.8

The small blepharoplast is removed a short distance in most individuals. The nucleus, compact and deeply staining, is oval or compressed. The undulating membrane and free flagellum are absent or very rudimentary. The aflagellar extremity is rounded; in some of the larger forms it becomes more pointed.



The morphological appearances in the first dog and in the sub-inoculations from this are identical; no 'long' forms were seen in any animal.

### *Diagnosis*

The animal reactions of this trypanosome, though limited in number, indicate a degree of pathogenicity not seen in *T. nanum*, while the absence of any long form would appear to negative *T. dimorphon* or *T. confusum*. We consequently associate this trypanosome with *T. congolense*.

The subject was a small wire-haired fox terrier 'Jock' which accompanied one of us throughout his stay in the country. The history of this dog is of interest as showing the resistance enjoyed by some dogs towards infection by *Glossina*.

He was in contact with *Glossina morsitans* or *Glossina palpalis* almost daily from September, 1907, to February, 1908, and again from April to the end of May, and during the last week in August, 1908. In March, 1908, he became weak and listless, with pallid membranes and a temperature up to 103°, but no trypanosomes could be demonstrated, and a rapid recovery was made. From the middle of October he again manifested signs of illness, commencing here with marked oedema of both ears; a few days later he had a temperature of 102°, rapid respirations, nose dry and warm, and visible membranes pallid. Trypanosomes were first seen on October 31st, and on only three other occasions during the next fortnight of daily examinations. He appeared to improve from November 24th, and no trypanosomes were seen after November 12th. He was left at Pretoria apparently improved, but Dr. Theiler tells us he rapidly went off and died, showing many trypanosomes shortly after our departure.

A dog (No. 9) was inoculated on October 31st with six drops of blood from 'Jock.' The temperature became irregular from the eighth day, but no trypanosomes were seen in the blood until November 21st: they were present in the glands from the tenth day following inoculation. Trypanosomes remained present, but always scanty (one or two to a cover-glass, rarely one in ten fields), up to death on November 27th.

Dog No. 16 was inoculated subcutaneously on November 27th with 1.5 c.c. heart blood of Dog 9. Trypanosomes were never seen in the blood of this animal up to its arrival in Pretoria; but gland puncture on December 20th showed infection to have resulted.



*Morphology of the 'Jock' Trypanosome*

In the original and the sub-inoculated dog the parasite seen corresponds in every way with that already described in 'Dip' and in 'Balungu'; to this description we have nothing to add.

## TRYPANOSOMIASIS OCCURRING IN A NATURALLY INFECTED PIG

On October 7th we received from a European in Abercorn a young male pig for experimental purposes. This animal was one of a litter born in May at Abercorn, where within fifty miles *Glossina morsitans* or animal trypanosomiasis have never been seen or suspected, and where there is no traffic to introduce it. On arrival this animal was footsore and dull, but this was ascribed to the conduct of the native who drove it to us a distance of fifty miles in two days. It was kept in our camp and appeared quite healthy until October 31st, when the native in charge reported it sick. On examination it was recumbent, semi-comatose, breathing heavily, visible membranes injected, considerable oedema of the prepuce and a temperature of 102°. Trypanosomes were swarming in the blood, and the animal died six hours later.

*Autopsy* commenced immediately. Oedema of prepuce; all subcutaneous vessels markedly congested; body fat plentiful.

The same marked congestion in all mesenteric veins: liver and kidney slightly congested. Spleen considerably enlarged, soft and friable, with Malpighian bodies prominent. Some excess of blood-tinged fluid in the pericardium; coronary vessels of heart greatly distended. Ante-mortem clots in both auricles and ventricles. Blood dark and clots readily; lungs pale.

All lymphatic glands swollen, soft and excessively haemorrhagic.

The picture presented was that of very acute haemorrhagic septicaemia.

We are quite unable to say where infection was derived; the sudden death indicated rapid disease due to a local infection after arrival in our camp. This could not be entirely excluded owing to existence of *Stomoxys*, *Hematobia* and *Tabanus* in small numbers; but the pig had been in intimate association with only Ox 1, which had an infection with a trypanosome (Chunga) morphologically distinct, and which only became infected five days prior to the pig's death. From the fact of its indisposition on arrival we might surmise a previous infection from some as yet unknown source in Abercorn. There the pig had been in intimate contact with sheep, the histories of which are always uncertain owing to the possibility of traffic in

these animals, and which, as we have already noted, may be a great source of danger.

An enquiry into the morphology and animal reactions of the trypanosomes met with, and a comparison with those on our camp previously, might aid towards a possible solution. We are only able to give a summary of the few reactions obtained prior to the arrival of the strain in Pretoria; it being our main object to preserve it for experimentation in suitable surroundings.

### *Experimental.*

All inoculations made direct from the pig.

RAT 9.—October 31st. Inoculated intraperitoneally with 5.0 c.c. blood. No trypanosomes were ever seen, and this animal arrived at Pretoria in good condition.

GUINEA-PIG 11.—October 31st. Inoculated intraperitoneally with 5.0 c.c. blood. Trypanosomes were never seen. It died on December 19th, probably from exposure. There were no indications of trypanosomes on post-mortem.

DOG 10.—October 31st. Inoculated intraperitoneally with 5.0 c.c. blood. During two weeks of observation the temperature remained normal, and organisms were not seen on blood or gland examination. On the march they were not encountered at various examinations. Death took place from pneumonia on January 3rd. There were no indications of trypanosomes on post-mortem.

GOAT 6.—October 31st. Inoculated subcutaneously with 5.0 c.c. blood. The temperature rose to  $106.4^{\circ}$  on the fifth day, and for the remaining ten days of observation assumed the type met with in Broken Hill.

Trypanosomes, one in five fields appeared on the eighth day, were absent on the ninth and tenth (temperature low), were seen two in quarter cover-glass on the eleventh (temperature  $107.2^{\circ}$ ), absent the twelfth (temperature low), and were again present on the next thermal elevation on the 13th day. There consequently appeared to be a connection between temperature and presence of organism.

This goat travelled well, and arrived in Pretoria in good condition. Trypanosomes were not seen in the peripheral blood on the journey, but a gland puncture on December 21st showed them still present. At Pretoria (January, 1909) a rabbit, guinea-pig and a sheep were inoculated, and on April 26th Dr. Theiler informs us that the original goat is still alive, that the sheep is infected, but the rabbit does not show anything.

### *Morphology of the 'Pig' Trypanosome*

The trypanosomes present at death of the original host were so numerous that no details of movement could be made out; this more especially, too, since they were agglutinating. In the goat they could be seen crossing the field without difficulty, giving the impression of a gliding motion.

In stained preparation. (1) *Pig*. The trypanosomes measure from  $11.5$  to  $17.9 \mu$  in length and from  $1$  to  $1.8 \mu$  in breadth. The

majority of forms seen were in a state of active multiplication, so accounting for the average length of  $15.75 \mu$ . The mean length of single individuals was  $13.15 \mu$ . In these latter the cytoplasm was relatively clear and homogeneous, the blepharoplast some  $0.5 \mu$  from the rounded or bluntly angular extremity; the nucleus rounded and rather loose in texture being centrally placed. There is a slightly better development of the rudimentary undulating membrane than is usual in these 'tadpole' trypanosomes, but practically no free flagellum.

In divisional forms the aflagellar extremity was more pointed, and vacuoles were seen in the cytoplasm of many. The undulating membrane was better developed, amounting almost to that common in *T. lewisi*, and a free extremity to the flagellum of 2 or  $3 \mu$  was seen in several individuals. Where this portion could not be designated 'free,' this extremity of the body or the ectoplasm was drawn out more than in the smaller single forms.

(2) *Goat*. In this animal the trypanosomes which appeared measure from  $14.5$  to  $17.25 \mu$  (average  $15.97 \mu$ ) in length and  $1.5$  to  $2.6 \mu$  in breadth (average  $1.83 \mu$ ). The following is the mean of a series of measurements:—

Extremity to Blepharoplast	Blepharoplast to Nucleus	Nucleus	Nucleus to flagellar extremity
1.4	4.6	2.47	7.5

No free flagellum was seen, but, as in the pig, the flagellar extremity of the body was in some cases drawn out. The cytoplasm stained rather pink, and granules or vacuoles were infrequent. The aflagellar extremity is rounded, but more elongated than in the tadpoles of *T. congolense*. Dividing forms (up to  $17.25 \mu$ ) were present, and in these there is some approach to a very small undulating membrane, which in the solitary individual is as rudimentary as in 'tadpoles.'

### Diagnosis

From the morphology of this trypanosome it would appear that we are dealing with either *T. congolense* or *T. nanum*. The animal experiments are limited, but a dog, a rabbit (at Pretoria), a guinea-pig and a white rat remained healthy after large doses of heavily infected



blood, which, from the acuteness of the pig's sickness, might be considered of a virulent nature.

Our observations are too limited to identify this trypanosome with certainty as *T. nanum*, which is characterised by negative features; but we incline to the opinion that future work will show they are closely related.

We can now consider the question of where this pig derived infection, and at once a local origin, a transmission from the Scotsdale strain which was being maintained, will suggest itself. *T. nanum* was not found locally and was unknown around Abercorn; and though its existence there cannot be denied, and is on some grounds to be suspected, it appears more probable that this pig acquired the disease after arrival at Kambole, where *Stomoxys* and *Haematopota* were common.

#### VII. EXAMINATIONS OF GAME

Wherever possible an examination was made of all wild animals ranking as game which we shot, or which had been killed in the vicinity of our camps by others.

In most cases this consisted of examining the blood taken from the ear or heart in fresh cover-glass preparation; but latterly gland juice, and sometimes also that of other organs, was also examined. In no instance was blood or other fluid centrifuged. Inoculations into small animals were only made from those showing flagellates and from a wart-hog and a buffalo shot by Dr. Yale-Massey close to our camp at Broken Hill. Dry slides were made from some, and were examined stained for the presence of other blood parasites such as piroplasma and spirochaeta. These have not yet been properly examined, but no findings are recorded up to date.

The majority of these animals were killed at considerable distances from camp, and several hours elapsed before examination; but not a few, as for example those shot on the march, came under the microscope within an hour.

Bruce has indicated that direct examination is of small value; but the positive findings of Dutton and Todd and ourselves show that peripheral organisms may be present. The ideal system, however, would be to establish temporary camps in various districts with a



plentiful stock of healthy animals carefully protected in fly-proof cages at hand, and to inoculate such immediately on the death of the game, or to convey citrated blood back with as little delay as possible. This would have to be carried out in both clean (fly-free) and tsetse infested districts; and it is one of the first problems in the etiology of trypanosomiasis that should be undertaken. In Northern Rhodesia, and elsewhere, considerable difficulties will be experienced owing to the non-pathogenicity of certain endemic trypanosomes towards the ordinary laboratory animals; it would appear almost imperative, therefore, that sheep and goats should be employed.

In addition to game, wherever possible all small animals such as mice, rats, moles, bats, snakes, lizards, birds, as well as crocodiles were examined. Trypanosomes were seen only in a species of bat; no blood parasites were observed in the crocodile.

The following is a list of the game examined. It has not been deemed necessary to publish the localities wherein each was shot; it is enough to say that they were obtained in both clean and fly-infested districts:—

Elephant	...	... <i>Elephas africanus</i>	...	...	...	...	...	2
Hippopotamus	...	... <i>H. amphibius</i>	...	...	...	...	...	1
Buffalo	...	... <i>Bos caffer</i>	...	...	...	...	...	1
Eland	...	... <i>Tragelaphus spekei</i>	...	...	...	...	...	2
Sable Antelope	...	... <i>Hippotragus niger</i>	...	...	...	...	...	1
Roan Antelope	...	... <i>Hippotragus equinus</i>	...	...	...	...	...	13
Zebra	...	... <i>Equus burchelli</i>	...	...	...	...	...	11
Hartebeest	...	... <i>Bubalis lichstensteini</i>	...	...	...	...	...	18
Sessaby	...	... <i>Damaliscus lunatus</i>	...	...	...	...	...	4
Waterbuck	...	... <i>Cobus ellipsirymnus</i> and <i>C. defasa</i>	...	...	...	...	...	9
Puku	...	... <i>Cobus vardoni</i>	...	...	...	...	...	34
Lechwe	...	... <i>Cobus licbi</i>	...	...	...	...	...	2
M'pala	...	... <i>Aepiceros melampus</i>	...	...	...	...	...	3
Reedbuck	...	... <i>Cervicapra arundinum</i>	...	...	...	...	...	30
Bushbuck	...	... <i>Tragelaphus scriptus</i>	...	...	...	...	...	4
Oribi	...	... <i>Oribia scoparia</i>	...	...	...	...	...	8
Wart-hog	...	... <i>Phacochoerus aethiopicus</i>	...	...	...	...	...	11
Bush pig	...	... <i>Potamochoerus chaeropotomus</i>	...	...	...	...	...	2
Duiker	...	... <i>Cephalopus grimmi</i>	...	...	...	...	...	1
Lion	...	... <i>Felis leo</i>	...	...	...	...	...	1

Of these 158 three only showed flagellates, in two they were recognised as trypanosomes; that in the third does not belong to this

genus, and will be described independently. All these animals were shot in districts infested by *Glossina morsitans*.

1. Bushbuck, *Tragelaphus scriptus*. A young male in good condition, shot November 10th, 1907, at N'tampwa, some forty miles south-west of Ndola in North-Western Rhodesia.

The heart blood was examined about one and a half hours after death, and trypanosomes about one in ten fields seen in fresh preparation. A white rat was inoculated at once with 2.0 c.cm. intraperitoneally, but never became infected and was killed by *T. congolense* eleven months later.

*Morphology of the trypanosomes.* In fresh preparation the movement was limited to the field. Stained, the following is the mean of ten measurements:—

Extremity to Nucleus	Nucleus	Nucleus to flagellar extremity
4.31	1.9	5.85

The picture, then, is one of a tadpole trypanosome, which may be associated with *T. dimorphon*, *T. confusum*, *T. congolense* or *T. nanum*. The failure to obtain this strain in the inoculated rat leaves its identity uncertain.

2. Hartebeest, *Bubalis lichtensteini*. An adult male in normal good condition, shot on the 8th December, 1907, about twenty miles north of Ndola. The heart blood was examined within two hours of death. Filaria present (they are apparently fairly common in this antelope), and two trypanosomes were seen in two fresh cover-glass preparations. A white rat was inoculated intraperitoneally with 1.5 c.cm., but never became infected. In fresh preparation the trypanosome was localised to the field and produced but scanty movement among the corpuscles. It was apparently short, not more than 15  $\mu$ , and did not possess a flagellum. We have been unable to find any organism in the dry films made from this animal.

Inoculations were made intraperitoneally into healthy dogs from the heart-blood of a buffalo (5.0 c.cm.) and a wart hog (5.0 c.cm.) shot by Dr. Yale-Massey close to our camp at Broken Hill. The buffalo belonged to a herd known to frequent tsetse areas in the locality. In neither case did the dogs become infected.

VIII. SUPPLEMENTARY NOTE TO OUR 'REPORT ON  
TRYPANOSOMIASIS OF DOMESTIC STOCK IN  
NORTH-WESTERN RHODESIA'

INFECTION BY *T. dimorphon*, pp. 104-112.

On our departure from Broken Hill all infected goats and sheep were left at the camp in charge of a native, but were kindly visited on one or two occasions by a European, who has informed us these animals all succumbed before the end of January, 1908.

Donkey, Case No. LV, page 108. This animal remained in splendid condition and indeed improved, and in March was sold at an enhanced price, despite the purchaser's knowledge of his history. He was alive and working well at the end of December, 1908, thirteen months after trypanosomes were seen in his blood. We have to thank the Administration for making arrangements for the regular inspection of this animal by the local Medical Officer.

The strain of *T. dimorphon*, derived from Case XXV, was taken away with us in guinea-pigs and rats, but owing to the shortage of these animals it was permitted to die out at Madona early in 1908.

INFECTION BY *T. vivax*, pp. 112-117.

We paid a hasty visit to Broken Hill in November, 1907, two months after our departure, and were then informed by the native in charge that sheep, Case XXXIX, had been one of several killed by a leopard the previous week. With the loss of this animal the strain disappeared.

Donkey, Case No. LVIII, page 114. This animal was repurchased by his original owner on return from leave, and taken to Chinsali, where we again examined him in May and August, 1908, without finding any signs of trypanosomiasis.

MORPHOLOGY OF THE CATTLE TRYPANOSOMES, pp. 118-123.

In a later publication we have discussed the nomenclature of these trypanosomes, and more recently we have emphasised the view that the Broken Hill *T. dimorphon* is identical with the original of Dutton and Todd, which is distinct from that strain later employed both at

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\* *Annals Trop. Med. and Parasit.*, Vol. II, No. 2.

Liverpool and Paris. We publish a series of photographs illustrating the morphology of this trypanosome (Plate III, figs. 1, 2, 3 and 4).

The impracticability of maintaining this strain is much to be regretted; it is possible that it may be again recovered at or near Broken Hill.

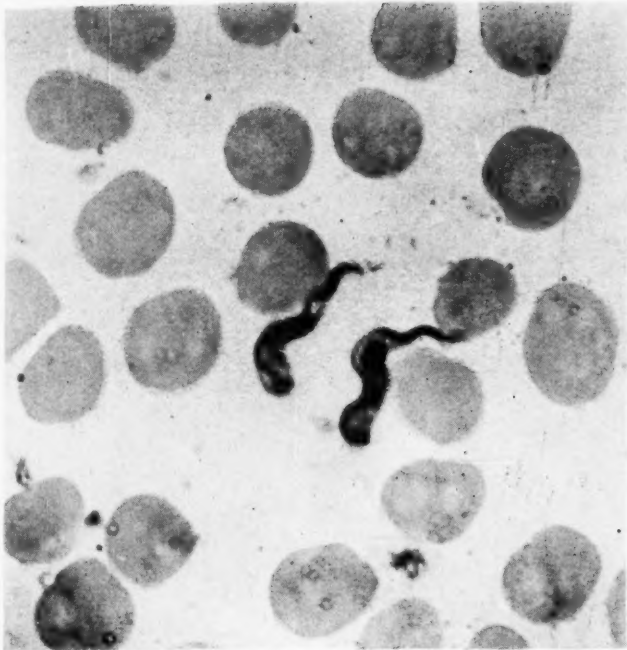
#### TRANSMISSION OF THE CATTLE TRYPANOSOMES, pp. 128-131.

In the present report we are able to adduce further evidence to corroborate our view that *Stomoxys* was responsible for transmission in the Broken Hill herd, which had not been exposed to *Glossina*.

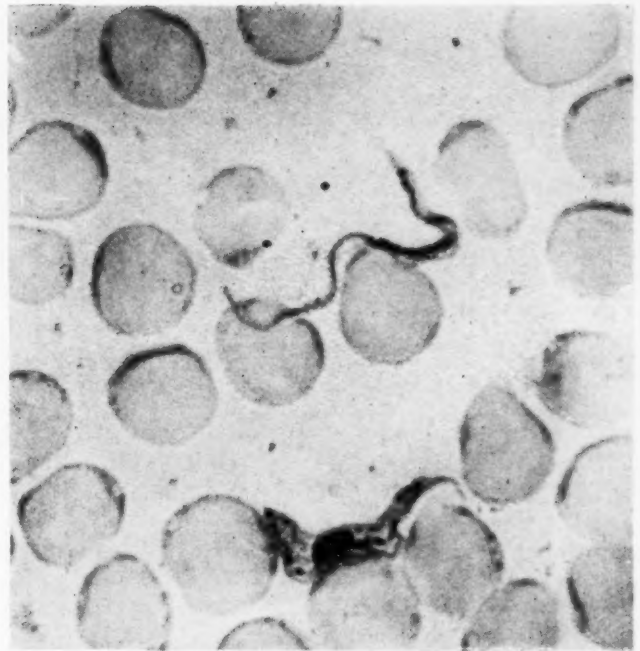
It may be recollected that two healthy oxen were exposed to *Glossina morsitans* on the road from Mwomboshi to Broken Hill, and that only three flies were seen to feed. Both these animals became infected on the eighteenth day with *T. vivax*. Respectively seven and eight days later they showed an organism morphologically identical with tadpole *T. dimorphon*.

It is most undesirable that this crude observation should be quoted as evidence of the infectivity of *Glossina*. Although these cattle were kept by themselves on arrival, they were not in fly-proof stables, and the possibility that they were attacked by *Stomoxys* on the farm cannot be excluded; indeed, the occurrence of a double infection would, *a priori*, indicate, especially in Case XLIV (one *G. morsitans* seen to feed), that *Stomoxys* should be incriminated. We would further point out that the diagnosis of the trypanosomes in these animals is based solely on the morphological appearances, and that no sub-inoculations were made. It will therefore be apparent that this also is open to grave question, and that the so-called double infection may in reality be but a manifestation of morphological variation in a single species, such, for example, as Wenyon and ourselves have noted in *T. nanum*.

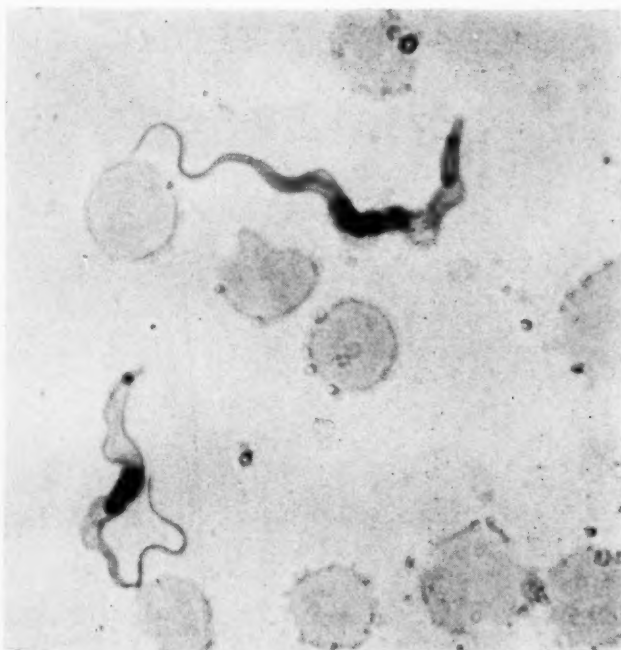




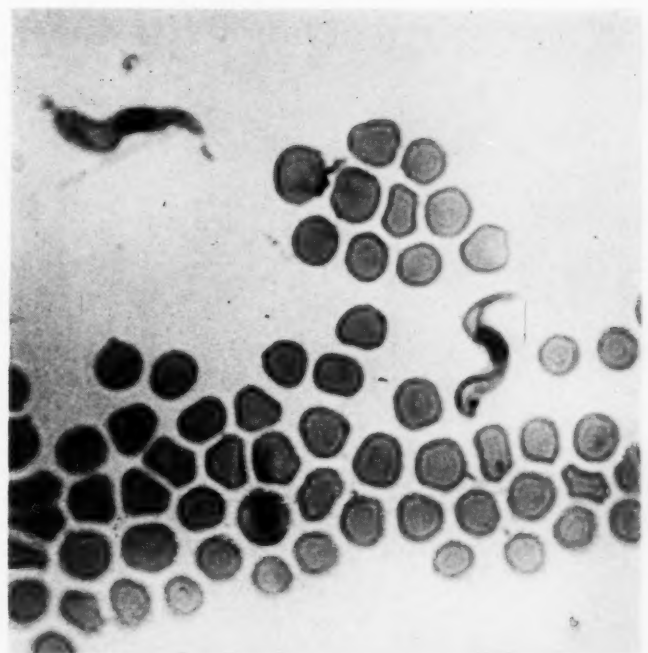
1. *T. dimorphon* (Dutton and Todd). Tadpole form in dog, from naturally-infected cow.



2. *T. dimorphon* (Dutton and Todd). Stumpy and long form in guinea-pig, from dog.



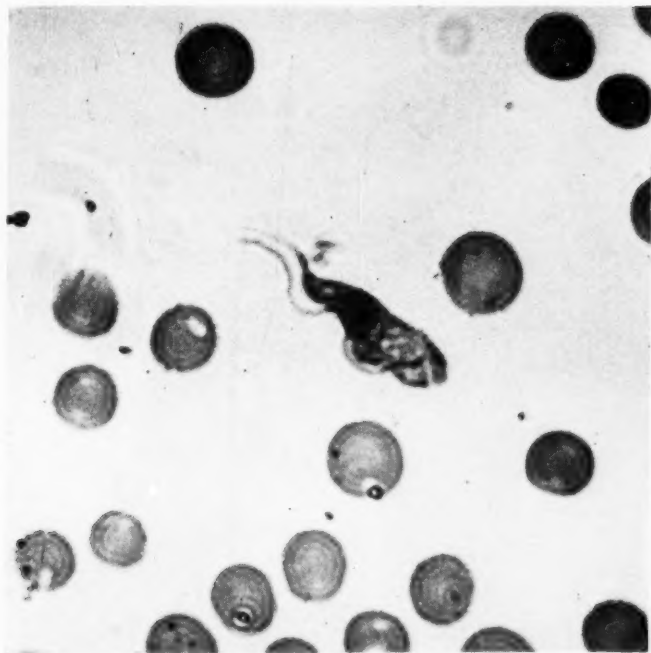
3. *T. dimorphon* (Dutton and Todd). Long form in guinea-pig, from naturally-infected cow.



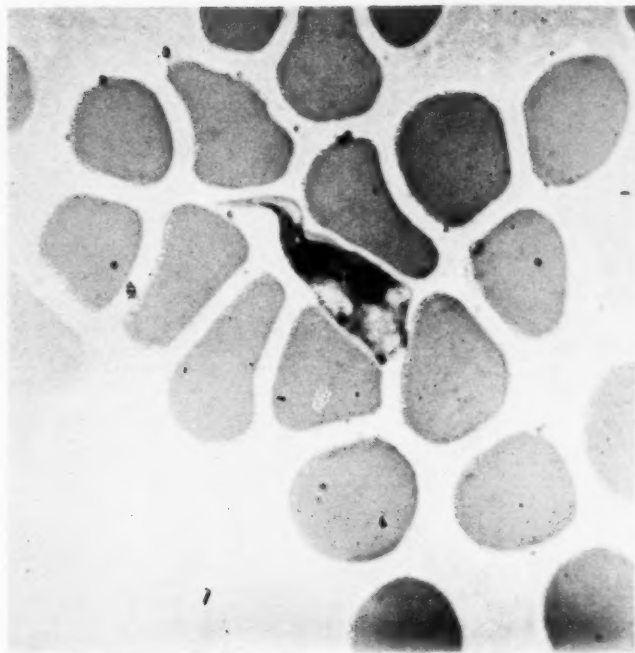
4. *T. dimorphon* (Dutton and Todd). Tadpole form in goat, from guinea-pig, showing long forms.

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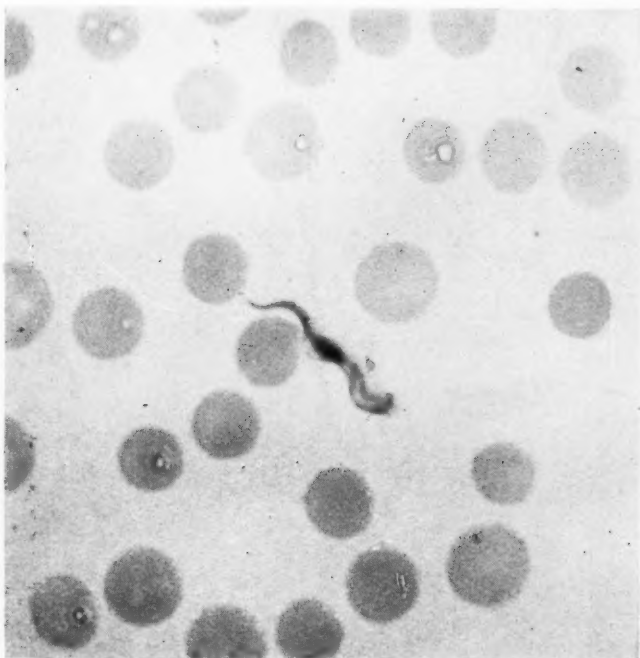
PLATE IV



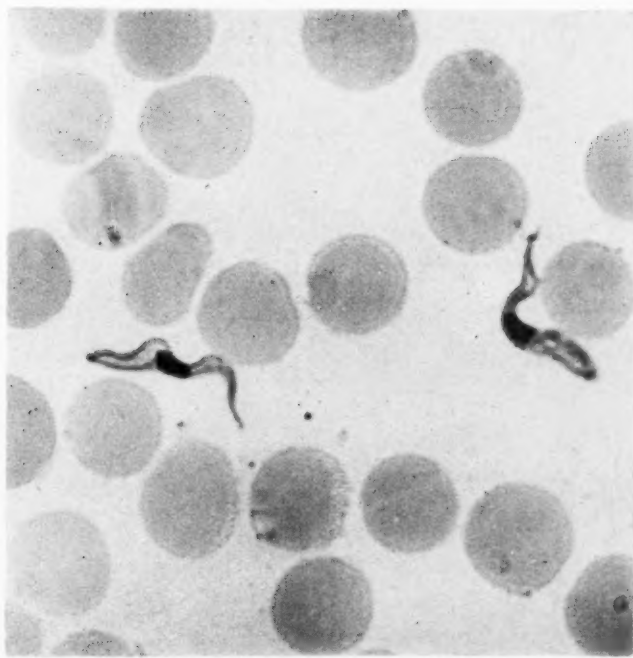
1. Ninamwenda strain in naturally infected cow.



2. Ninamwenda strain. Divisional form in guinea-pig, from naturally infected cow.

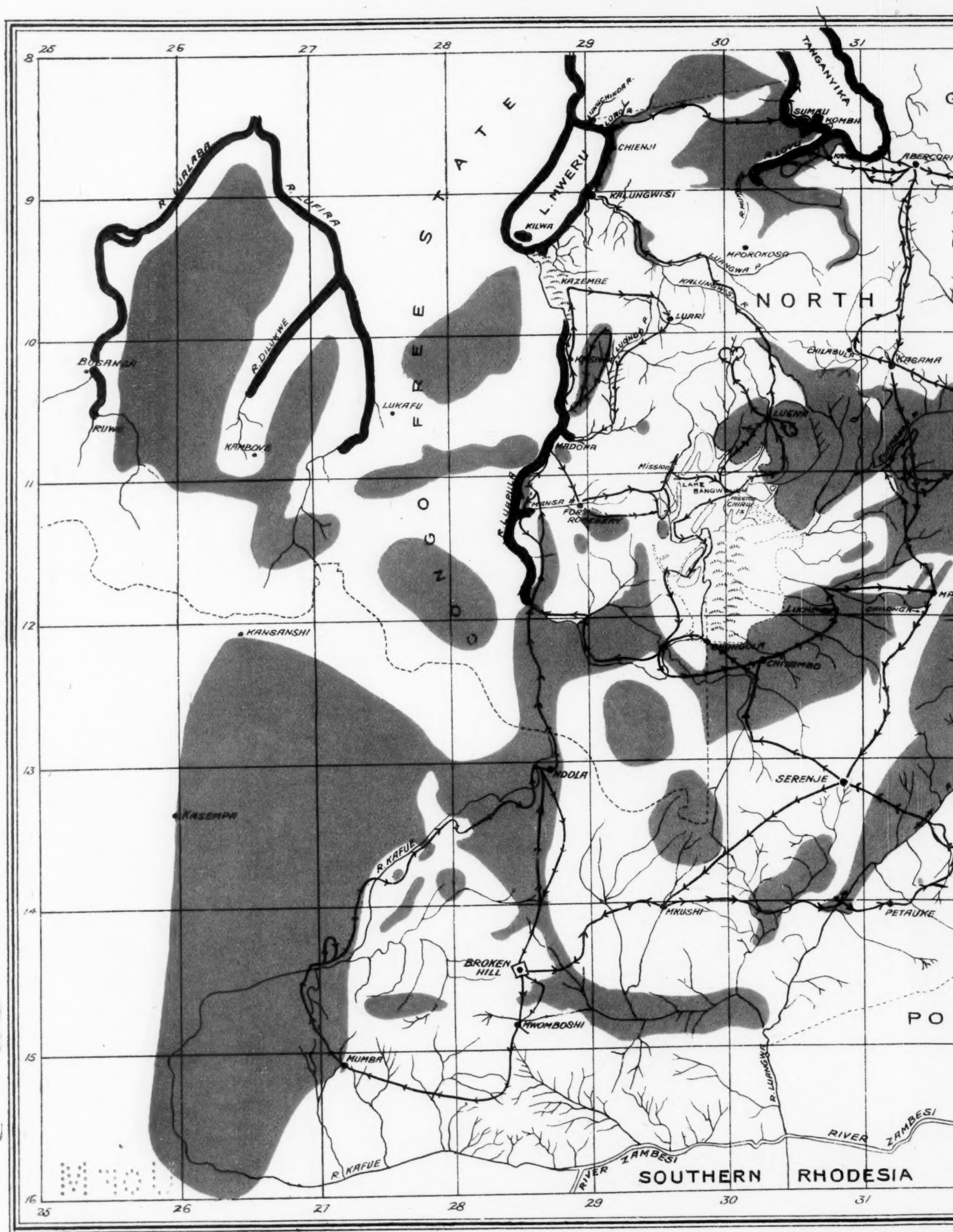


3. *T. congolense* (Laveran) (?). Balungu strain in naturally-infected cow.

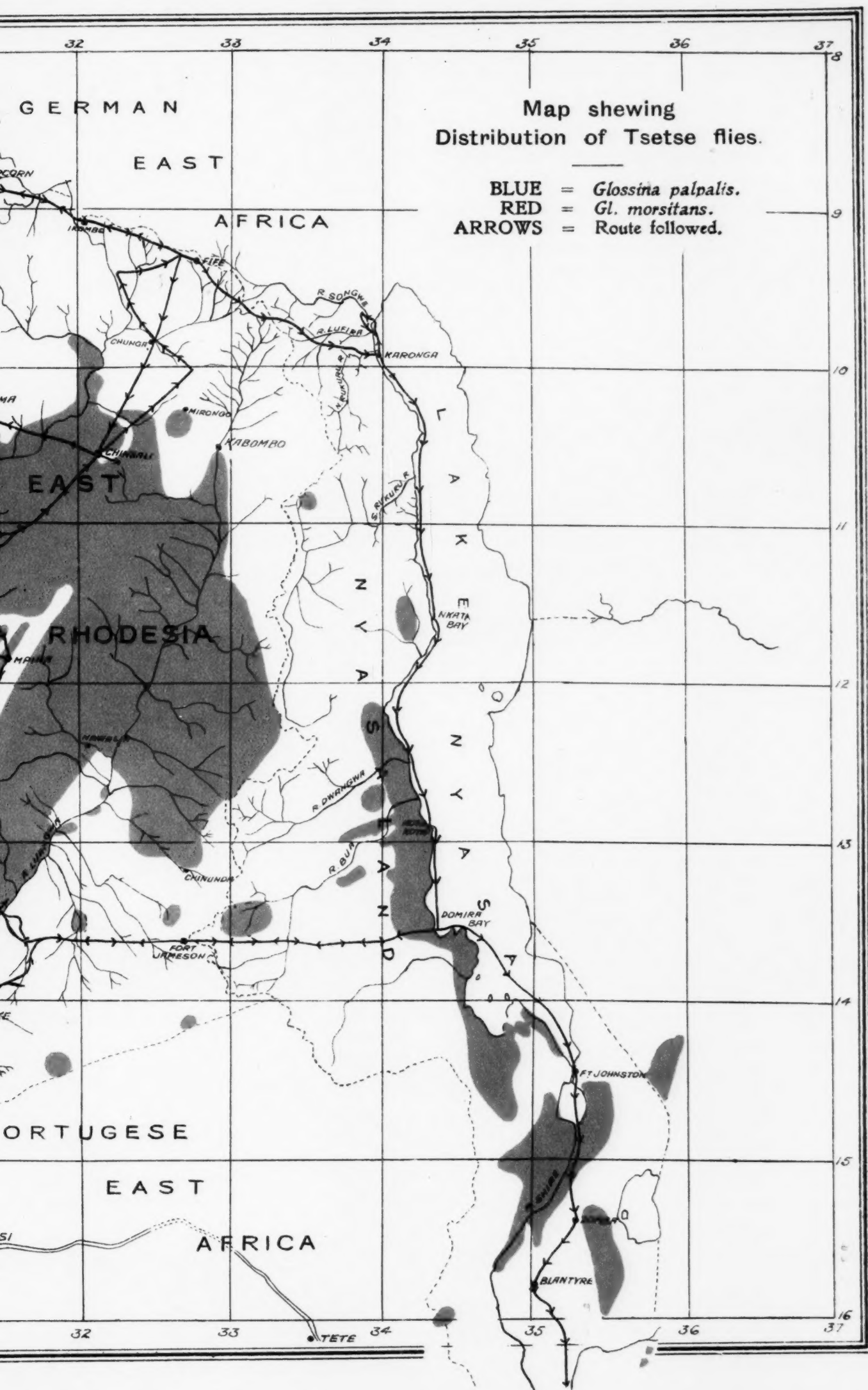


4. *T. congolense* (Laveran) (?). Wallace strain in naturally-infected dog.

U. S. N. M.









## SANITARY MEASURES AND MALARIA EPIDEMICS OF ATHENS

BY

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*(Received for publication 2 June, 1909)*

It is well known that for a good many years past, severe and widespread malarial fevers have afflicted the inhabitants of the two large districts of Athens, Pangrati and Vatrachonisi; and according to my studies since 1900 as well as the statistical information of the local doctors of the above two districts, there has for a long time been much suffering from malaria. The morbidity varied between 25 and 30 per cent., not counting the epidemic of the years 1885, 1886, etc.; but from 1901 to 1906, before putting in force the sanitary measures and during the epidemic years, it has ranged from 49.09 per cent. to 92.85 per cent.

This amazing prevalence of the epidemic has absorbed the entire attention of the League; and as soon as the League was formed, it entrusted me to explore the sources and causes of the malaria fever, and to ascertain by what means sanitary measures could be carried out for the above two districts of the city of Athens (whose inhabitants number 8,000).

The cause of the said epidemic, as I ascertained, was the Illisus river, in whose pools of stagnant and polluted waters, innumerable legions of Anopheline mosquitoes were hatching.

In these stagnant pools I observed, in 1901-1907, only one species of Anopheline, viz., *P. superpictus*, but in my research during the year 1908 I discovered a new species of Anopheline, as yet undescribed, different from, but close to *P. superpictus*; my opinion

being confirmed by Mr. Newstead, of the Liverpool School of Tropical Medicine, to whom I sent specimens for examination.

To remove the causes of the malaria, I proposed two ways: first, temporary works of sanitation to be repeated each spring, and secondly, the building of permanent works.

The second proposition was not approved for lack of sufficient funds, but temporary works were preferred, which consisted in the transformation of the branches and small tributaries of the Illisus river-bed, by filling them up, by excavation and removing rocks; so that the flowing water might be concentrated in a sloping narrow channel in such a way as to run free without an impediment.

The work done by me for the first time, in the year 1906, on account of its beginning late in the season, afforded small relief, because the Anophelines had already hatched before the work was commenced; in consequence of which the progress of malaria, as will be seen in the Table below, could not be prevented.

In the second year, 1907, the work was begun in the latter part of the month of May in Pangrati district, and a little later in a branch of the Illisus river, viz., from the Iton bridge of Vatrachonisi district up to Rizarios Seminary. The work contributed to a large extent to the reduction of the malaria, because in the Pangrati locality, where the work of sanitation began early in the season, the morbidity from malaria dropped to 2 to 3 per cent., whereas in Vatrachonisi district, where the work was commenced later in the season or in the beginning of summer, the morbidity was from 25 to 30 per cent.

In the year 1908, the third year of the sanitary measures, they were carried out on all the branches or rivulets of the Illisus river in time, i.e., before the summer season. The malaria was, as I have ascertained, greatly reduced; and as the League was informed by the local doctors, the morbidity caused by malaria fell to 1 per cent.

The sanitary measures in the above two districts, without making any use of preventives, such as quinine, etc., have proved of great value in suppressing malaria.

Confirmation of the splendid results in suppressing malaria of the above sanitary measures is plainly afforded, not only by the examination of the sick rate among little children, but by the examination of their spleens.



COMPARATIVE TABLE OF THE SICKNESS CAUSED BY MALARIA  
AMONG THE CHILDREN IN THE DISTRICTS OF  
PANGRATI AND VATRACHONISI.

*Before the Sanitary Measures.*

Number of Children	Summer	Infected	Sick rate
* 280 .....	1901 .....	260 .....	92·85%
200 .....	1902 .....	160 .....	80%
235 .....	1903 .....	192 .....	87·70%
180 .....	1904 .....	89 .....	49·09%
200 .....	1905 .....	185 .....	92·50%
<u>1095</u>		<u>886</u>	<u>80·90%</u>

*After the Sanitary Measures.*

Number of Children	Summer	Infected	Sick rate
† 301 .....	1906 .....	177 .....	58·80%
‡ 345 .....	1907 .....	73 .....	21·15%
§ 300 .....	1908 .....	8 .....	2·66%
<u>946</u>		<u>258</u>	<u>27·27%</u>

*Examination of the spleen of the above children in 1907.*

Of 186 children examined in April, 1907, 29, or 15·59 per cent., had enlarged spleen.

Of 345 children examined in November, 1907, 10, or 2·89 per cent. had enlarged spleen.

*Examination of the spleen of the same children in 1908.*

Of 290 children examined in April, 1908, 6, or 2 per cent., had enlarged spleen.

Of 300 children examined in November, 1908, none had enlarged spleen.

\* The statistics from 1901-1904 were made in collaboration with Professor Pezapoulos.

† The measures were taken late in the season.

‡ The measures were taken in time.

§ The measures were taken in time.

### CONCLUSIONS

Studying my statistics, I observe that the malaria for the period of five years before the undertaking of the sanitary measures reached 80.9 per cent.; after the period of three years, from the beginning of these measures, malaria among the children was reduced to 1 per cent.; consequently we come to the conclusion that the suppression of malaria, and the restoring to health of Pangrati and Vatrachonisi, are entirely due to the undertaking of sanitary measures in the river-bed of the branches of the Illisus river.

We can, therefore, wage war on malaria with good results in a country if we proceed to disinfect it as described.

The photographs show sections of the Illisus river before and after the operations.



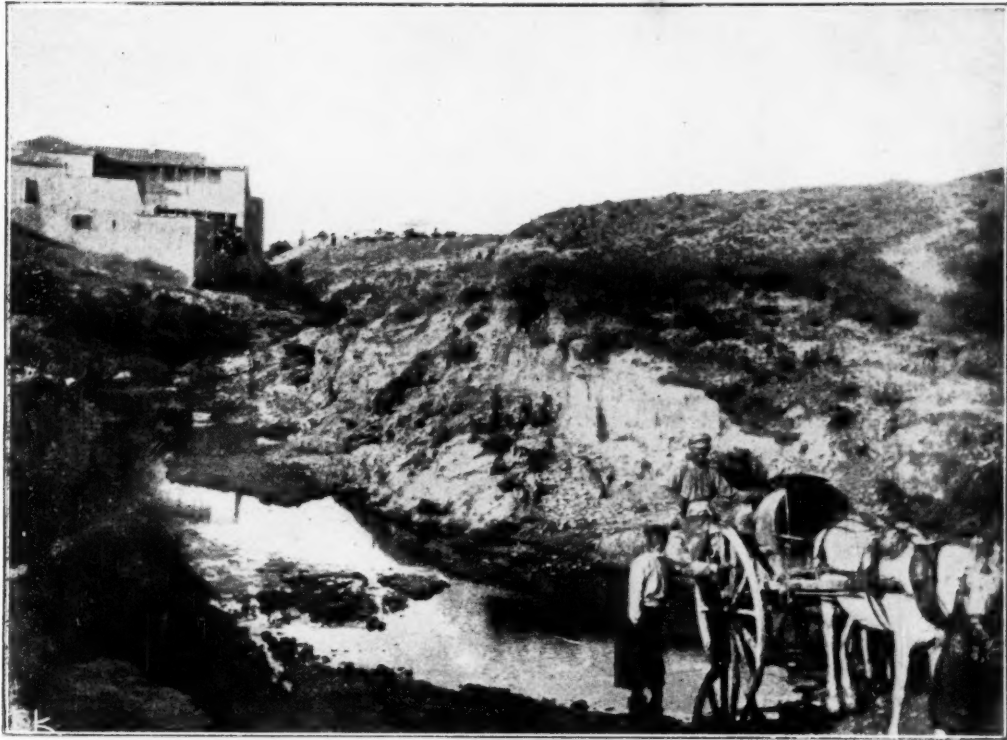
BEFORE OPERATIONS.



AFTER OPERATIONS.







BEFORE OPERATIONS.



AFTER OPERATIONS.





BEFORE OPERATIONS.



AFTER OPERATIONS.





# AN ACCOUNT OF A FORM OF SPLENOMEGALY WITH HEPATIC CIRRHOSIS, ENDEMIC IN EGYPT

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## INTRODUCTORY

All medical men who have had experience of native practice in Egypt are aware of the frequency of cases of ascites due to hepatic cirrhosis. Thus we find that such cases account for 4 per cent. of admissions to the medical wards of Kasr-el-Ainy Hospital, Cairo, as compared with 0.9 per cent. in the large London hospitals. This fact is striking, as it occurs in a country where the large majority of the inhabitants are Moslems and alcoholic excess is rare. Moreover, its prevalence in children, and peculiar clinical and pathological characters, easily distinguish it from the ordinary European variety.

Familiarity with the condition of the liver and spleen in such cases enables one to recognise the great prevalence of the disease in an earlier stage, before the onset of ascites. An enlargement of both these viscera is frequently noted in patients admitted for various complaints, and the liver may be felt in all stages of cirrhosis. Finally, it is a common experience to find splenic enlargement without obvious hepatic changes in patients who present no history nor signs of malarial infection.

The notorious difficulties of tracing hospital patients at home are as nothing compared with the impossibility of obtaining any reliable information whatever of the antecedent or subsequent histories of native patients seen in hospital. Only large experience

can partly remedy this defect, and the history and course of the disease must be gathered more from careful observation of patients admitted in various stages rather than from a series of individual cases followed from the commencement to the end.

### GENERAL ETIOLOGY

*Incidence.* Hepatic cirrhosis must be considered one of the common diseases which affect the native Egyptian, although much less prevalent than trachoma, ankylostomiasis and bilharziosis. A census of native patients in the medical wards of Kasr-el-Ainy Hospital showed that over 4 per cent. were admitted for ascites, and no less than another 16 per cent. had the disease in easily recognisable form, while an additional 7 per cent. had chronic splenic enlargement which in most instances represented the earliest stage of the disease.

This cirrhosis frequently complicates ankylostomiasis and pellagra, and is especially common when these two diseases exist in combination. This association, while striking clinically, has little direct relationship, for advanced cases of both diseases generally show no signs of cirrhosis during life or at autopsy. Further, ankylostomiasis and pellagra are the two commonest diseases on the returns from the medical side.

A truer idea of the prevalence of the disease is afforded by the results of a special routine examination of all native patients admitted to the surgical and eye sections (septic cases excluded). Of a total of 300 of all ages :

8 per cent. had splenic enlargement (chronic).

3·3 per cent. had enlargement of the liver and spleen.

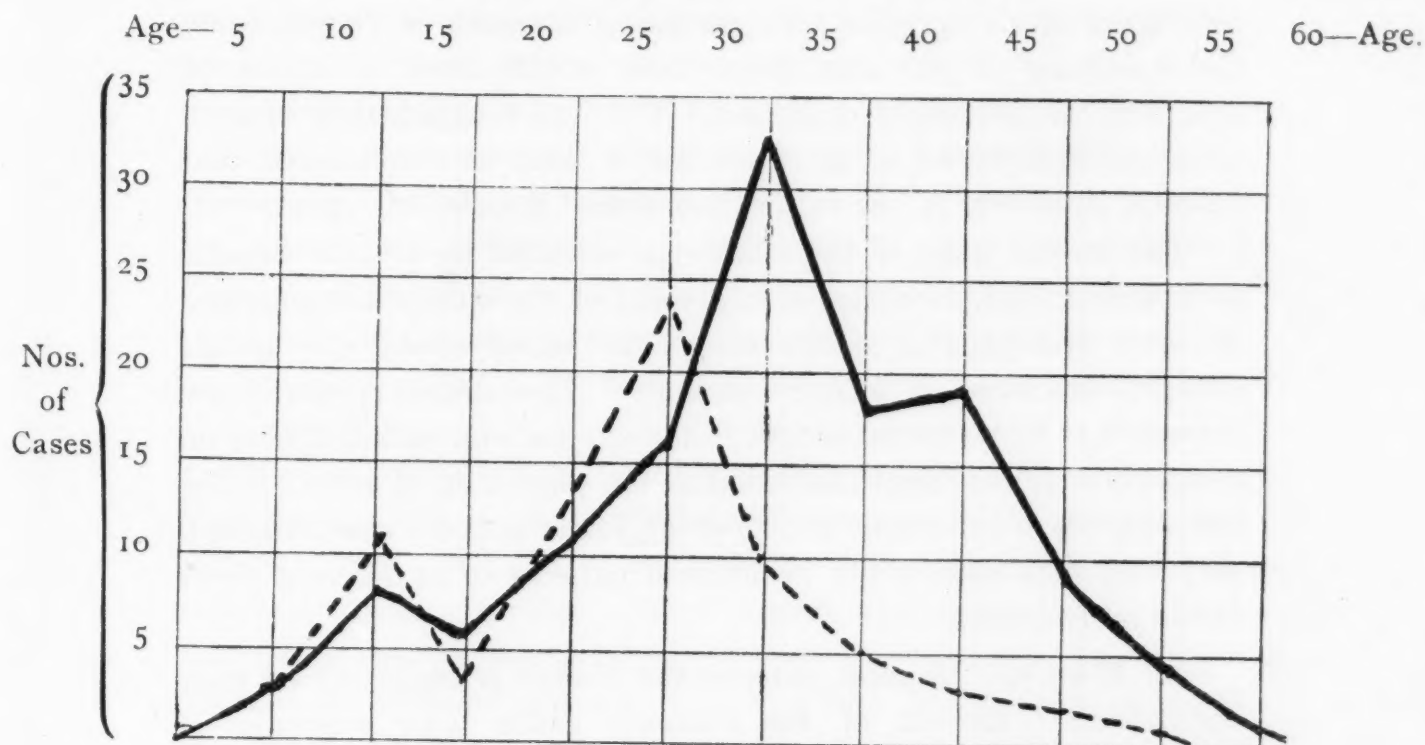
2·3 per cent. had definite cirrhosis with enlarged spleen.

This total is not large enough to show the incidence at each decade with accuracy, but the results so far bear out the age incidence recorded below. On this point an interesting investigation has already been begun by Dr. E. H. Ross, Medical Officer of Health for Cairo, who examined over 7,000 children under 16 years of age and found 6·8 per cent. with enlarged spleens.

*Age.* The appended chart shows the numbers and ages of cases admitted during two years, in two curves, the continuous line showing the age incidence among patients with well-established disease and

the interrupted the ages of those admitted with hepatic ascites. It will be seen on comparison that this disease is most common after twenty-five, and that the onset of ascites is generally postponed for five or more years. The irregularity of the fall in the latter group is probably due to the difficulty in estimating the age, as native patients are quite ignorant on this point.

CHART SHOWING AGE INCIDENCE



*Sex.* According to the above return the disease is equally prevalent in either sex. The special census of hospital patients showed a decided predominance in the male sections, but this may be explained by the small numbers of young female adults admitted of the age at which the disease is most common. A family history was rarely obtainable, but little reliance can be placed on such statements, which only special village investigation can establish or disprove.

#### SYMPTOMS

Although in its later stages this disease presents a characteristic picture, its onset is unattended by any constant symptom except a slow painless enlargement of the spleen. Upon examination this

organ is felt slightly enlarged, of a firmer consistence than normal, descending one or two finger-breadths below the costal margin on inspiration. Some degree of anaemia is the rule rather than the exception among the native population owing to the prevalence of ankylostomiasis, but is not a special feature of these cases.

Inquiry among patients in a later stage of the disease showed that no symptom other than abdominal enlargement and discomfort had been noticed in 55 per cent.; 29 per cent. described an onset with fever of an irregular type persisting a month or longer, while the remaining 16 per cent. dated their trouble from an attack of dysentery or prolonged diarrhoea. The exact significance of such intestinal disturbance is uncertain, but it must be remembered that amoebic dysentery is one of the commonest diseases of Egypt.

The second stage of the disease is indicated by definite hepatic involvement, with increased enlargement of the spleen, accompanied by local and general symptoms. The blood shows the special changes described in a later section. The clinical picture now presented is by no means identical in every patient, as will appear on comparison of the cases illustrated in the photos A, B and C. The following set of symptoms are, however, found in every case, although they may vary in intensity in different patients or at different times in the same patient.

(1) *Wasting.* In most patients the disease is suggested by their appearance. Instead of the extreme pallor with retention of fat which characterises ankylostomiasis, or the emaciation with localised pigmentation distinctive of pellagra, we find a moderate anaemia with more or less wasting. The combination of marked anaemia with wasting in an Egyptian generally indicates the co-existence of ankylostomiasis and cirrhosis.

(2) *Fever.* The great majority of cases show slight fever of an irregular type. In 63 per cent. this did not exceed 37·5, but in 28 per cent. there were excursions up to 38, and in 9 per cent. this temperature was exceeded. Occasionally patients may show high fever for some weeks due to an exacerbation of the disease itself and not to any complication. The temperature, as in Case C, may show a double remission in the twenty-four hours, similar to that described in Kala-azar.



(3) *Local symptoms and signs.* A dragging pain in the left side due to the enlarged spleen is common. Discomfort after meals and tenderness over the hypochondrium is generally present at some stage, caused by commencing perisplenitis and perihepatitis. In advanced cirrhosis, congestion of the stomach and fixation by adhesions may set up a chronic dyspepsia which the patient seeks to relieve by inducing vomiting after a heavy meal. Haematemesis is not common, but may be the first symptom of the disease and be accompanied by other signs of portal congestion. These complaints are often illustrated by the scars of cauteries, setons, etc., with which the patient has sought to relieve his pain. See photos A and D.

Upon examination the abdomen is seen to present the characteristic shape illustrated in Plates VIII, IX, X. There is an outward expansion of the lower ribs, a widening out of the costal angle, and a general enlargement of the upper part of the abdomen—often with separation of the recti muscles—the result of the pressure exerted by the enlargement of the liver and spleen, which may indeed form visible swellings.

The condition of these viscera varies within considerable limits, and is influenced by the duration and severity of the disease, and possibly also by the age of the patient. The liver is felt uniformly enlarged, smooth at first and of firmer consistence than normal. Later this enlargement is attended with fibrotic contraction which gives it an irregular granular surface. The largest liver noted in this stage measured six inches downwards from the costal margin in the nipple line. With increasing cirrhosis the organ shrinks, but is rarely reduced to less than its normal weight. When the spleen is much enlarged the hepatic changes are obscured, since this organ is displaced upwards and to the right.

The spleen attains its greatest dimensions when the disease is advancing rapidly, more especially in young subjects below the age of 20. Two illustrative cases are shown in Plates VIII and X. In such patients it may reach some distance beyond the umbilicus, and the diaphragm with the thoracic viscera are displaced upwards. It may overlap the liver if the latter be also much enlarged.

With the gradual development of hepatic cirrhosis the spleen becomes much harder and may shrink somewhat. Thus the state of the liver can generally be inferred from the consistence of the spleen,

for if the latter be hard, even if only projecting a finger breadth below the costal margin, hepatic changes are certain to be advanced. Adhesions may prevent the descent of the spleen, and in some cases, where the liver has been markedly cirrhotic, the former organ could not be felt on abdominal examination.

The duration of this stage may apparently be indefinite. The longest interval noted before the appearance of ascites was fifteen years. Many mild cases may show no further symptoms, and the progress of the disease be arrested or the patient be carried off by some intercurrent illness. (Plate XI.)

The disease, however, attracts most notice when the hepatic cirrhosis is followed by ascites with its attendant miseries. This serious event may be due to the gradual obliteration of hepatic vessels, but in many cases the history suggests that a fresh infection, acting on an already cirrhotic organ, may be responsible. Evidences of portal congestion, such as nausea, vomiting, haematemesis, melaena and haemorrhage from the bowel or from piles may precede its onset. Rapid emaciation follows.

At this stage the patient presents the familiar picture of cirrhotic ascites. (Plate XII.) The pinched features and wasted limbs offer a striking contrast to the greatly swollen abdomen. The effect of the high intra-abdominal pressure is seen in the protuberant umbilicus and network of dilated superficial veins which return blood from the oedematous legs into anastomoses with the thoracic vessels. Jaundice is rare except as a terminal event. Upon palpation the cirrhotic liver can usually be felt in the epigastric angle, and the enlarged spleen recognised by 'dipping.' Most cases with ascites require tapping on admission, and this has to be repeated on an average every ten days, about eight kilos being withdrawn on each occasion. After removal of the fluid these organs can be better investigated, when the liver is more often found enlarged than contracted, and the spleen hard and of considerable size. The heart is displaced upwards, the urine may show traces of albumen, and congestion of the bases of the lungs with bronchitis usually follows. These latter signs may be an indication of heart failure and be complicated by the development of hydrothorax.

The duration of this stage is considerably shorter, and may be reckoned in months instead of years; the consequent prognosis is

much the same as in the alcoholic variety. The records of fatal cases show that death occurred on an average four months after the appearance of ascites; the longest interval was four years. Occasionally one meets cases where the patient has been temporarily or permanently cured. Thus two patients were tapped and remained free from ascites for three and a half and twelve years respectively. A recurrence in the latter case proved fatal, and at the autopsy no sign of syphilitic disease was discovered. Operation—omentopexy—has not so far given encouraging results, perhaps owing to the advanced state of the disease at the time.

The immediate cause of death was commonly hepatic insufficiency, the patients gradually passing into a comatose state—occasionally with jaundice. Lung complications and heart failure from exhaustion account for most of the remainder.

#### PATHOLOGICAL ACCOUNT OF THE DISEASE

A disease, characterised as this is, by initial fever, a chronic course, and a group of symptoms referable to slowly progressive changes in the liver, with either concurrent or consecutive changes in the spleen, exhibits many points of resemblance to Kala-azar. We have accordingly, during the past four years, examined the blood, spleen-pulp, and liver tissue during life, from such cases as illustrated the many clinical features in typical fashion.

Before proceeding to summarise the results of these observations, it may at once be said that we have never found in material taken from spleen or liver any parasites with the characters of the Leishman-Donovan body.

The blood has been examined by us in upwards of forty cases. The majority of these were admitted to hospital for the relief of ascites, cirrhotic change in the liver with splenic enlargement being already well established. The degree of anaemia is sometimes extreme, only 1,330,000 red corpuscles per cubic mm. being recorded in one of our cases. The average number of red corpuscles per cubic mm. in the forty cases examined was 2,635,440. The red corpuscles exhibit very considerable variations in size in the more advanced stages of the disease, although the discoid form is usually preserved. Polychromatophilia is frequently observed, chiefly



affecting the larger corpuscles, but the occurrence of nucleated red corpuscles is a very rare phenomenon.

With regard to the white cells, a definite leucopenia has always been found during what may be called the 'hospital phase' of the disease. Thus, the average leucocyte count in the series of cases examined was 4,503 per cubic mm., and the percentage proportions of the main varieties of leucocytes present, calculated from a series of differential analyses, gives the following figures:—

Polymorphonuclear neutrophiles .....	62·84
Lymphocytes .....	25·26
Large lymphocytes and hyaline cells .....	5·50
Eosinophiles .....	6·40
	<hr/>
	100·00
	<hr/>

Mast-cells are usually present, but seldom exceed the normal limit of 0·5 per cent. Neutrophile myelocytes were noted in a small proportion of the cases, but never exceeded 0·5 per cent.; for the sake of simplicity, therefore, these two classes of cells have been omitted from the composite table. It will thus be seen that the polymorphonuclear cells are relatively diminished, while the proportions of the other classes, and particularly of the large hyaline cells and eosinophiles, are distinctly increased. A certain degree of eosinophilia is of such frequent occurrence in Egypt, as the result of Bilharzial or Ankylostomal infections, that this feature of the blood formula may be at once discounted.

The bone-marrow has always been found more or less profoundly affected. That of the ribs is almost always diffuent, its colour varying with the degree of anaemia. The femur always manifests an active transformation of its marrow; in some cases this is of a reddish gelatinous character, and in others it is of deep red colour and firm consistence, resembling in appearance that of pernicious anaemia. In both situations the hyaline non-granular elements of the marrow are very notably increased, the majority of the cells being of the dimensions of a large lymphocyte with pale staining nucleus of simple spherical form. The relative reduction of the granular cells of the marrow, particularly of the neutrophile variety, is sometimes a marked feature, and is most noticeable in



marrow taken from the ribs. The marrow is also frequently the seat of congestion and haemorrhages. Nucleated red corpuscles are by no means abundant, and evidences of nuclear activity in any of the types of marrow cells are very rarely met with.

Numerous examinations of the spleen-pulp, which was either withdrawn during life, or obtained shortly after death, have been carried out, the result, as regards parasites, being, as above stated, uniformly negative. The material withdrawn from the spleen-pulp during life consists almost entirely of red corpuscles with a considerable number of lymphocytes. Very few of the large mononuclear phagocytes of the spleen are found in the contents of a syringe introduced into the enlarged and indurated organ so frequently accompanying the condition. In films made post-mortem, however, the latter class of cell occurs in considerable numbers and, both in films and sections, instances of phagocytosis in this type of cell are common. The presence of considerable numbers of granular cells (eosinophiles as well as neutrophiles) which occur in post-mortem preparations of the spleen-pulp has been frequently noted, fragments of such cells being often found in the macrophages of the spleen. To a certain extent, however, the assemblage of the granular cells in the spleen-pulp may be an ante-mortem phenomenon.

*Condition of the liver.* The comparatively frequent occurrence of cirrhosis of the liver in various degrees, with the usual morbid changes which are associated with this condition, is a fact which soon impresses itself on the attention of any one engaged in post-mortem work in Egypt. After the exclusion of types of cirrhosis which owe their origin to alcohol, tertiary syphilis or Bilharziosis, there yet remain a very considerable majority of all the cases of cirrhosis encountered for which some other explanation must be sought. With regard to alcoholic cirrhosis, it may be said at once that its occurrence amongst a largely Moslem population is extremely rare. Tertiary syphilis also does not appear to manifest itself amongst Egyptians in the form of gumma-formation and extreme cirrhotic change in the liver so frequently as amongst Europeans, and lastly, Bilharzial cirrhosis is not only comparatively rare, but when present is so characteristic in appearance that it can be easily differentiated. An analysis of the post-mortem records of the Kasr-El-Ainy Hospital, Cairo, for the last four years reveals the presence

of cirrhosis of the liver in some degree, exclusive of the types above mentioned, in no less than 9.9 per cent. of 1,430 autopsies recorded. Of this proportion, 4.5 per cent. may be said to have died as a direct result of the secondary changes induced by cirrhosis, exhaustion after repeated recurrences of ascites being probably the most frequent cause of death. In the remainder, cirrhosis of the liver was discovered incidentally, death in these cases resulting from a great variety of other diseases. In those cases in which the disease was more or less directly responsible for death, this occurred in the large majority of cases at about thirty-five years of age. Exceptionally, however, the same changes were encountered in subjects of over sixty years of age. The most typical morbid appearances were met with in young subjects dying between the ages of seven and twenty-one years. Approximately one-third of these cases was associated with some enlargement of the liver; in the remaining two-thirds, the organ was either normal or slightly reduced in size.

The cases in which cirrhotic changes in the liver were accidentally encountered were of the most diverse character. The type of cirrhosis met with under these circumstances has been of the fine diffuse variety, and not such as to induce much, if any, external alteration of the organ. With few exceptions, there is, in these cases, a slight general enlargement of the whole organ, as well as a moderate enlargement of the spleen. Microscopically, the evidences of cirrhosis are slight, a certain amount of cellular fibrillated tissue mixed with lymphocytes surrounding groups of lobules. In a typical case seen at this stage, and undisturbed by any coincident disease, the liver cells were for the most part large, swollen and highly granular. Their nuclei, stained by the Romanowsky method, were paler than usual, and each contained several nucleoli, which, by their depth of staining, contrasted strongly with the remainder of the nucleus. Here and there, minute isolated foci of necrotic appearance, surrounded by collections of small mononuclear cells, were met with, resembling generally those seen in the liver in typhoid fever infections. Careful search made both in films and sections for parasites always yielded negative results.

In cases where death was directly attributable to the results of long-established cirrhosis, the picture presented by the liver is very different. The organ in these cases is generally reduced in size,

though never extremely so. In the majority of cases, old adhesions due to perihepatitis exist between the convexity of the liver and the diaphragm, such adhesion being sometimes complete and universal. The characters of the liver generally fulfil the picture of a multilobular cirrhosis, the nodular projections on the surface being always small, firm, and closely set. In section, the colour is generally yellow or yellowish brown, but, as in other varieties of cirrhosis, many variations in tint are often seen in the various groups of isolated lobules.

Examined microscopically, persistent islands of hepatic tissue of irregular size, mostly rounded in shape, are separated from one another by extensive bands of connective tissue of somewhat varying character. The tracts of hepatic tissue contain large, universally granular liver cells; these have lost the more or less regular ramifying arrangement in relation to lobules—indeed no definite lobules are visible even in the largest persistent tracts.

The liver cells vary considerably in size, the larger frequently containing two nuclei, as if compensatory hepatic regeneration is in progress. The liver cells are frequently vacuolated or show other signs of degeneration than the granular character already mentioned. They sometimes exhibit a very marked 'vesicular' degeneration. In such areas, the hepatic cell nuclei are reduced in number, and those which persist, stain more faintly than is the rule elsewhere. The cells referred to have not a definitely fatty appearance, but suggest that degeneration products of the protoplasm have been dissolved out in great part, leaving only a hazy, reticulated residuum. The tracts of connective tissue separating the hepatic islands are densely cellular. Between the connective tissue fibrillae are multitudes of small lymphocytes. Polymorphonuclear cells, some of which are eosinophilous, occur fairly frequently amongst them. In these tracts, hepatic cells, single or in small groups, are found, showing pressure and other changes. Considerable numbers of small capillary vessels, with thin walls, are also found in these tracts. Where the lymphocytic infiltration is less dense, one sees that spindle-shaped fibroblastic elements largely compose the tissue. There is a comparative absence of the increased duct formation common in other varieties of hepatic cirrhosis.



*Condition of the spleen.* In the entire series of cases under consideration, enlargement of the spleen is the result. While splenic enlargement from other causes is of course frequently met with, it may be affirmed generally that distinct and sometimes great enlargement of the spleen is more regularly associated, in Egypt, with cirrhosis of the liver than with conditions unconnected primarily with this organ. As might be anticipated, the greatest degree of enlargement is found in cases where the cirrhosis is of the more advanced and contracted type just described. In several such cases, the weight of the spleen has been between 1,000 and 1,250 grammes, and in one case (that of a girl aet. fourteen with advanced cirrhosis) the weights of the liver and spleen were identical, viz., 1,450 grammes. Apart from such exceptional instances, however, the average weight of the organ has been found to be 450 grammes.

In consistence, the spleen is firm, frequently, indeed, quite hard, and presents a uniformly and deeply congested pulp in which the Malpighian bodies are generally only detected with difficulty.

Apart from the facts already stated in connection with the examination of the splenic tissue in films stained by the Giemsa or Romanowsky methods, the examination of sections of splenic tissue has yielded few results of any significance.

These may be summarised as follows:—

- (1) Hyperplasia of the lymphocytic elements of the pulp.
- (2) General increase of the connective tissue. This occurs either in the form of a definite increase of the compact fibrous trabeculae of the organ, or, in the case of the larger spleens, as an infiltration of spindle-shaped cells diffusely distributed throughout the entire pulp.
- (3) Distension and congestion of the vascular sinuses and frequent interstitial haemorrhages.
- (4) Active phagocytosis on the part of the macrophages towards red corpuscles and leucocytes. The occurrence of intracellular pigment from the former source, is commonly noted.

*Condition of the intestine.* In view of the lesions reported in the intestine in cases of Kala-Azar, the condition of the bowel has always been carefully scrutinised. It is not possible, however, in our opinion, to uphold the association of any definite lesion of the intestine with the condition in question, notwithstanding the clinical fact that



intestinal symptoms are very commonly a feature of the disease in the earlier part of its course. The intestine, in about a quarter of the cases examined, has presented various lesions. Some of these had all the characters of dysenteric ulceration either in an active or chronic form. Others showed an enterocolitis, affecting principally the lower part of the ileum and the entire colon, the inflamed surface being covered with a thin, irregular membranous exudation. In a few cases, multiple small oval ulcers of the colon were present. Their longer axes, generally less than one cm. in length, were transverse to the long axis of the bowel, and their edges were slightly raised, soft and crateriform. Microscopical examination of such lesions has, so far, yielded negative results, as regards the presence in them of any unusual parasites.

#### CRITICAL SUMMARY

Whatever the ultimate explanation of the condition dealt with in this paper may be, we feel that it constitutes a clinical and pathological entity the result of some infective agent of which we are as yet ignorant. The possibility that either Bilharziosis or Ankylostomiasis can have any causal relationship with this disease cannot, in the light of our statistical, clinical and pathological evidence, be admitted. Bearing in mind the condition of the blood and bone-marrow which characterises the disease, the deduction seems reasonable that, if it be produced by an infective agent, this is more likely to be found of protozoan than bacterial nature. In this connection, the recent discovery by one of us (A. R. F.) of large numbers of a parasite having the closest resemblance to that of Kala-Azar, and probably identical with that found in the Delhi Sore and Aleppo Boil, occurring in ulcerated papillomatous lesions on the limbs of Egyptian fellaheen, is not without significance. Dr. Bitter, Bacteriologist to the Government Department of Public Health, Cairo, has also found similar bodies in a lesion of this nature. It must be borne in mind, further, that our numerous attempts to find parasites in the internal organs have generally been carried out on patients already far advanced in the disease. Kala-Azar, while closely resembling the condition which we have described in certain respects, differs from it in others. Thus, the febrile onset, chronic course, presence of

intestinal symptoms, the aspect of the patient in the advanced stages, the condition of the blood, bone-marrow and spleen, are very closely similar in both affections. Kala-Azar, however, in the following particulars, apart from certain clinical features, differs from the condition to which we have called attention:—

(1) The presence of a crusted papular eruption or of ulcers on the limbs, containing the parasite.

(2) The presence of intestinal ulcers, chiefly in the colon, also containing the parasite in small numbers.

(3) The tendency to noma and local areas of gangrene, as well as to internal haemorrhages.

(4) The condition of the liver. In sporadic Kala-Azar, according to Rogers' observations, the liver showed marked cirrhotic changes in four out of forty-eight cases examined post-mortem, while slight degrees of fibrosis were observed in a certain additional number. In the condition we are describing, however, hepatic changes in various degrees form an integral part of the malady at different stages, and are constantly met with. Again, the type of cirrhosis met with is portal in character and not intralobular, as described by Rogers for Kala-Azar. And lastly, 'the persistence of the parasites in the advanced cirrhotic stage of the organ,' as noted by Rogers in Kala-Azar, is a feature which stamps a distinctive character on the liver in this affection.

For several reasons also we are unable to accept the view that chronic malaria can be held responsible for this class of case. The comparative rarity of malaria in Cairo may be gauged from the fact that not more than one or two cases are seen at hospital in the course of a year. Further, in the small proportion of cases of splenomegaly and cirrhosis accompanied by distinct fever there were no recurrent paroxysms, nor was the condition ameliorated by even considerable doses of quinine. Other reasons, founded on the pathological appearances, are the following:—

(1) The entire absence of any signs of malaria parasites (particularly the 'gametes' of former aestivo-autumnal infections) in our numerous examinations of the blood, and more particularly, of the spleen-pulp, in these cases.

(2) The absence of pigmented leucocytes in the circulation or in the viscera.

(3) The absence of visceral pigmentation in those situations commonly so affected in malaria.

(4) The character of the changes in the bone-marrow.

(5) The advanced degree and coarse character of the cirrhotic changes in the liver. It is, in our opinion, extremely doubtful whether malaria *of itself*, however severe or prolonged, can give rise to such pronounced cirrhotic change.

The differentiation of this disease from, or the determination of its possible relationship with, other forms of splenomegaly, such as chronic splenic anaemia, Banti's disease, and that found in marasmic infants suffering from gastro-enteritis, presents very considerable difficulties. To these, and closely allied to the last form, must be added a very similar but incompletely investigated condition, known under the name of 'Ponos,' and met with in certain islands of the Greek Archipelago.

That all these conditions, including that with which we have to deal in Egypt are referable to intestinal infection or toxæmia, is in the highest degree probable; and the view, so frequently urged by Continental writers, that certain of them (*viz.*, the splenomegaly of marasmic infants, chronic splenic anaemia, and Banti's disease) may all represent chronologically different stages of essentially the same disease, may ultimately be found to be true. But, until the essential identity and continuity of this prolonged process has been more regularly and constantly established, it appears necessary to retain the name 'chronic splenic anaemia' for what must be regarded, from the point of view of this hypothesis, as the intermediate stage of the process.

The condition which we describe is thus seen to differentiate itself at once from splenic anaemia by the regular coincidence of hepatic changes which, up to a certain stage, advance, *pari passu*, with those in the spleen, and taking the lead in the later stages, become largely responsible for the train of events leading to a fatal issue.

There remains the question of its identity with, or relation to, Banti's disease. It may at once be said that the existing records of the latter disease—comparatively restricted in number—do not permit of any differentiation between the two conditions. For the present, therefore, we may be content to remark that, closely comparable, if

not identical forms of splenomegaly with hepatic cirrhosis occur both in the South of Europe and in the northern part of the Egyptian Delta .

We have endeavoured to ascertain, hitherto with indefinite result, whether this condition also exists in those countries to the west of Egypt forming the African littoral of the Mediterranean, and also in Syria. It will probably be found not to be limited in distribution to Egypt, and indeed, may elsewhere at present be confused with, or classified under, other forms of splenomegaly, such as that occasioned by malaria.

It is with the object of emphasising its distinctive character, its prevalence in Egypt, and of drawing the attention of others at work in the Near East to the condition, that this paper has been written.

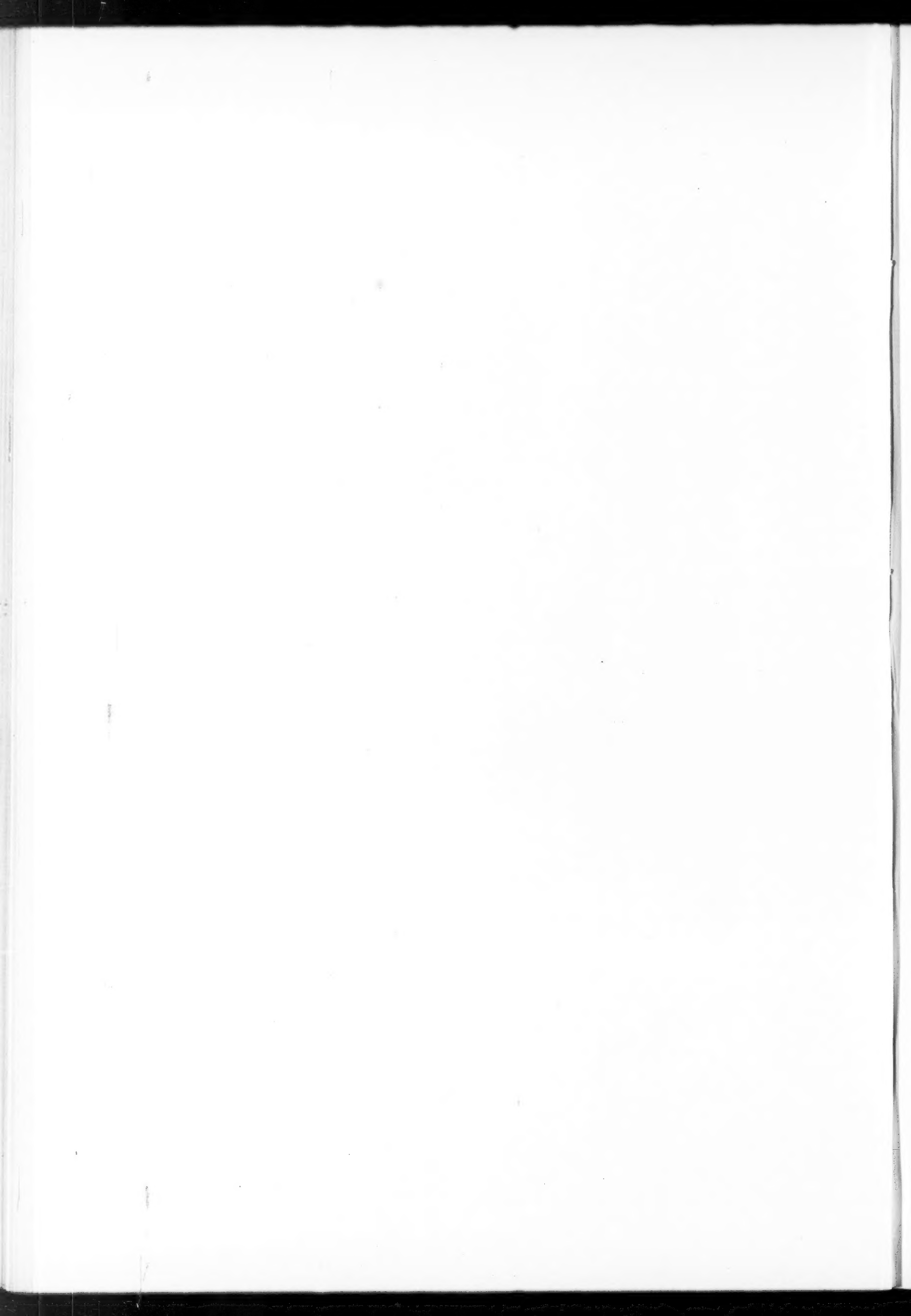
#### PLATES VIII-XII

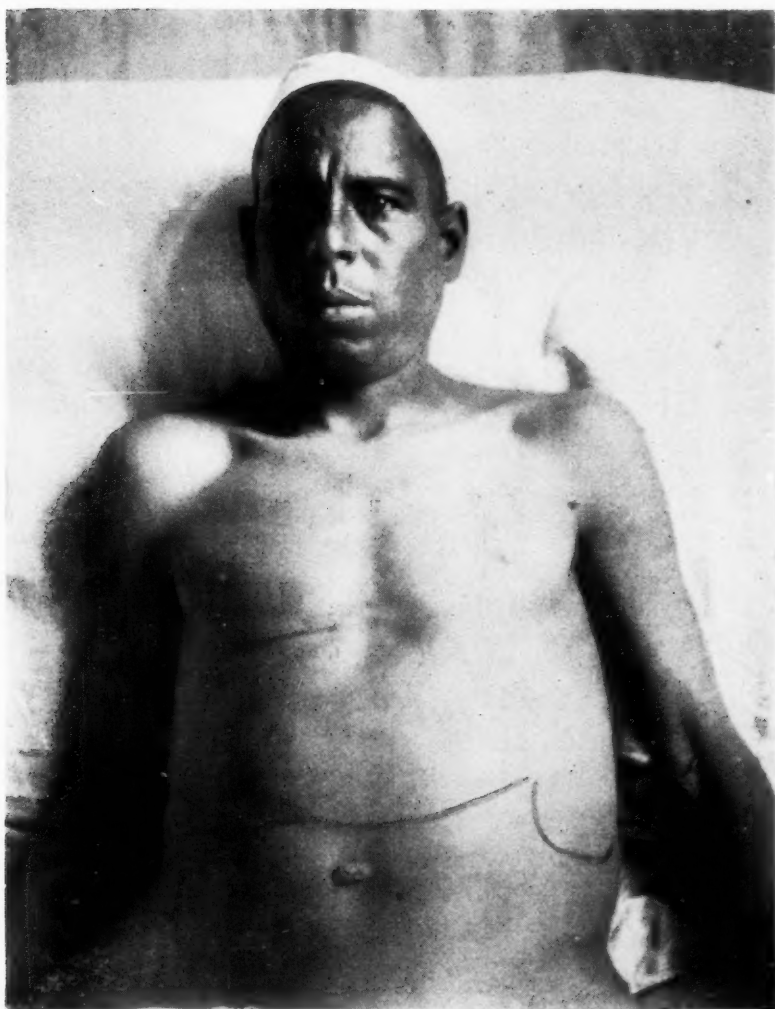
NOTE.—The outlines of the viscera in the cases illustrated were outlined with aniline pencil before photographing.



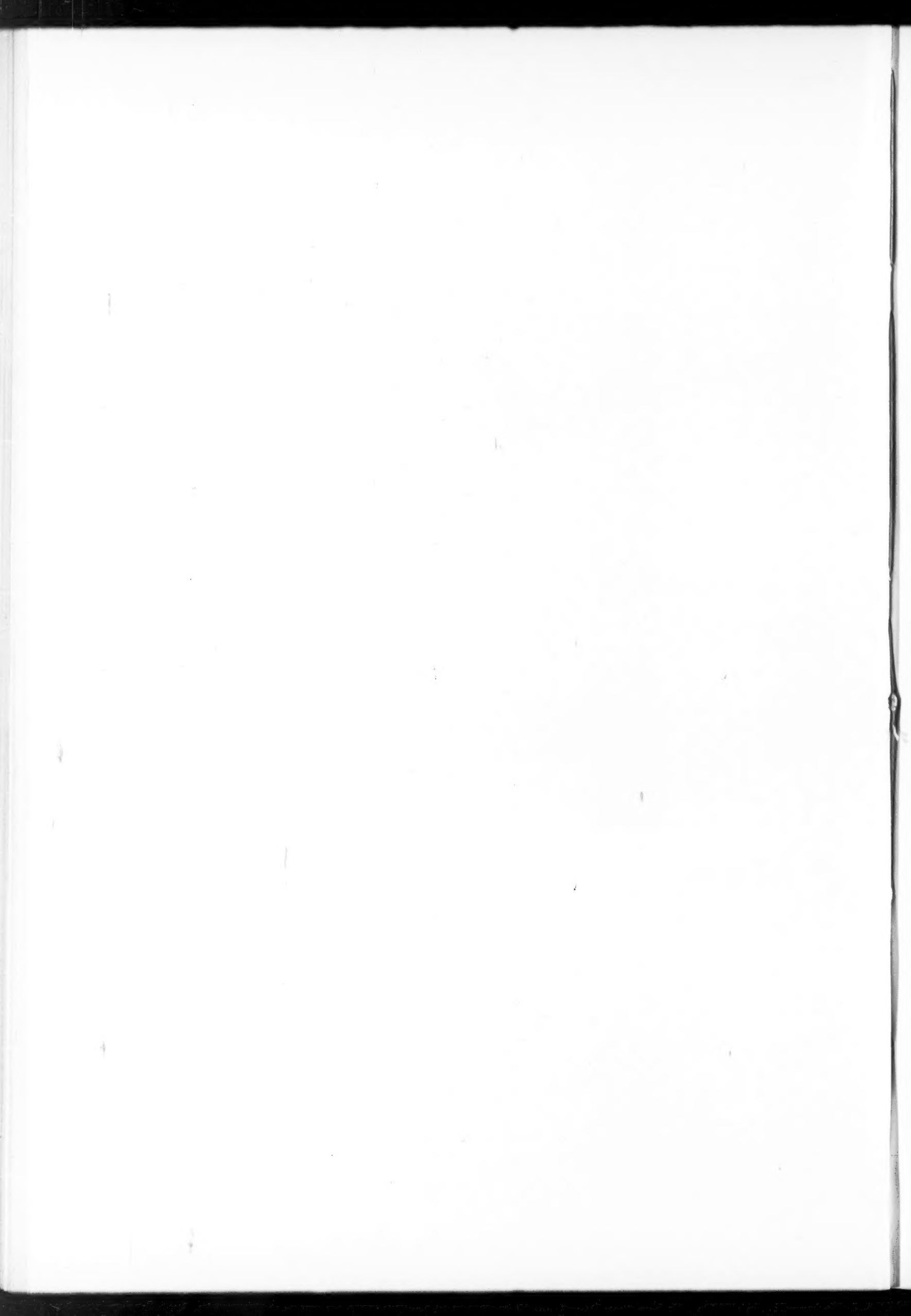


CASE A.—Male: aged 13. History of splenic enlargement and local pain for one year. No fever. Spleen punctured: no parasites found. Blood count: Red corpuscles, 3,960,000; White, 4,157.





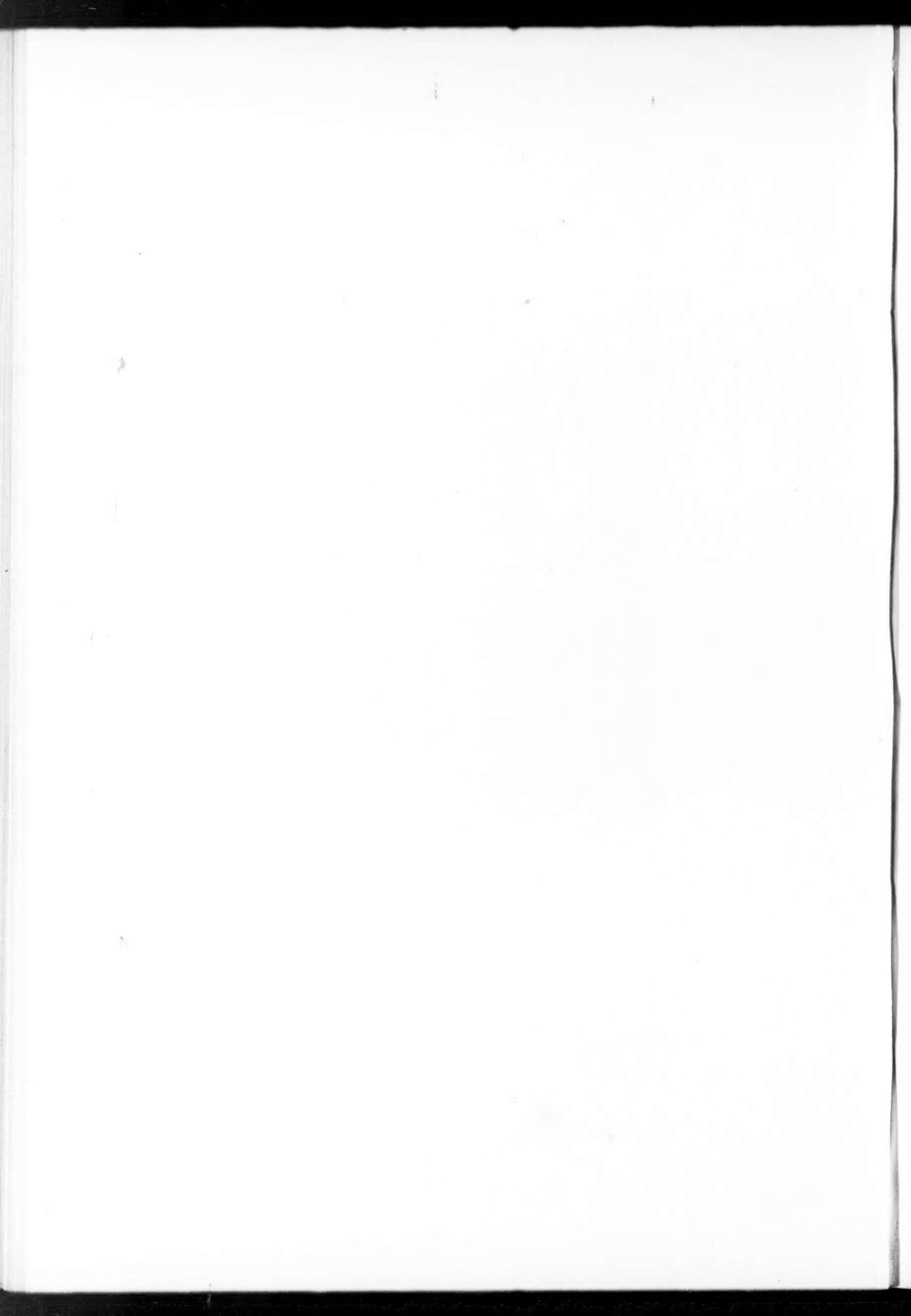
CASE B—Male: aged 25. No special symptoms noticed by patient, duration of illness unknown. Temperature normal. Liver especially enlarged and hard. Chronic type; had wasted.





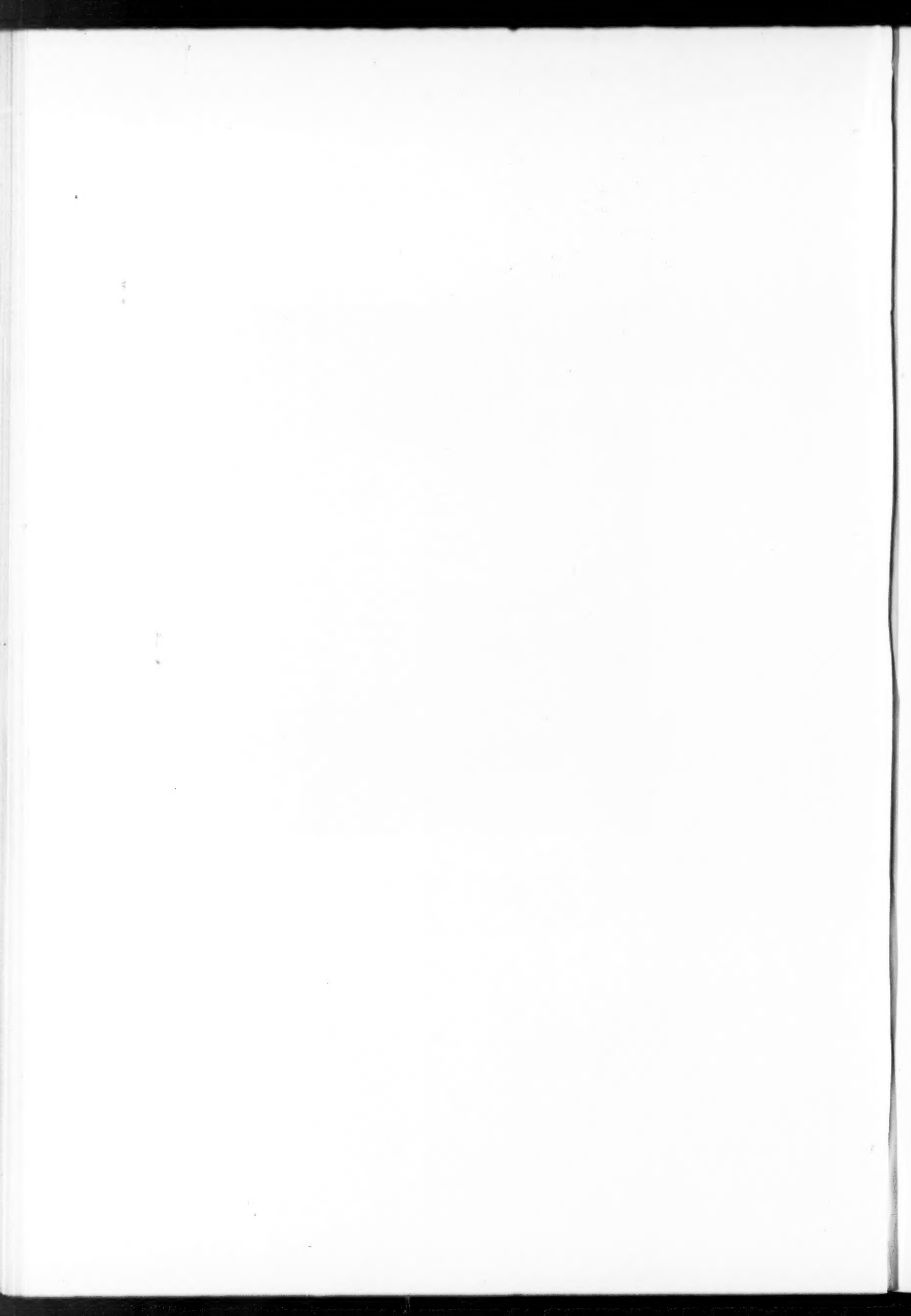


CASE C —Male: aged 22. Illness began three years ago with dysentery; has got much worse the last eight months. High remittent fever,  $39^{\circ}$  C. on admission, continuing weeks. Abdomen distended by splenic tumour; liver slightly enlarged, displaced upwards and tender. Blood count: Reds, 2,896,000; White, 6,200. Spleen punctured three times with negative findings. Sub-acute type, rapid emaciation.





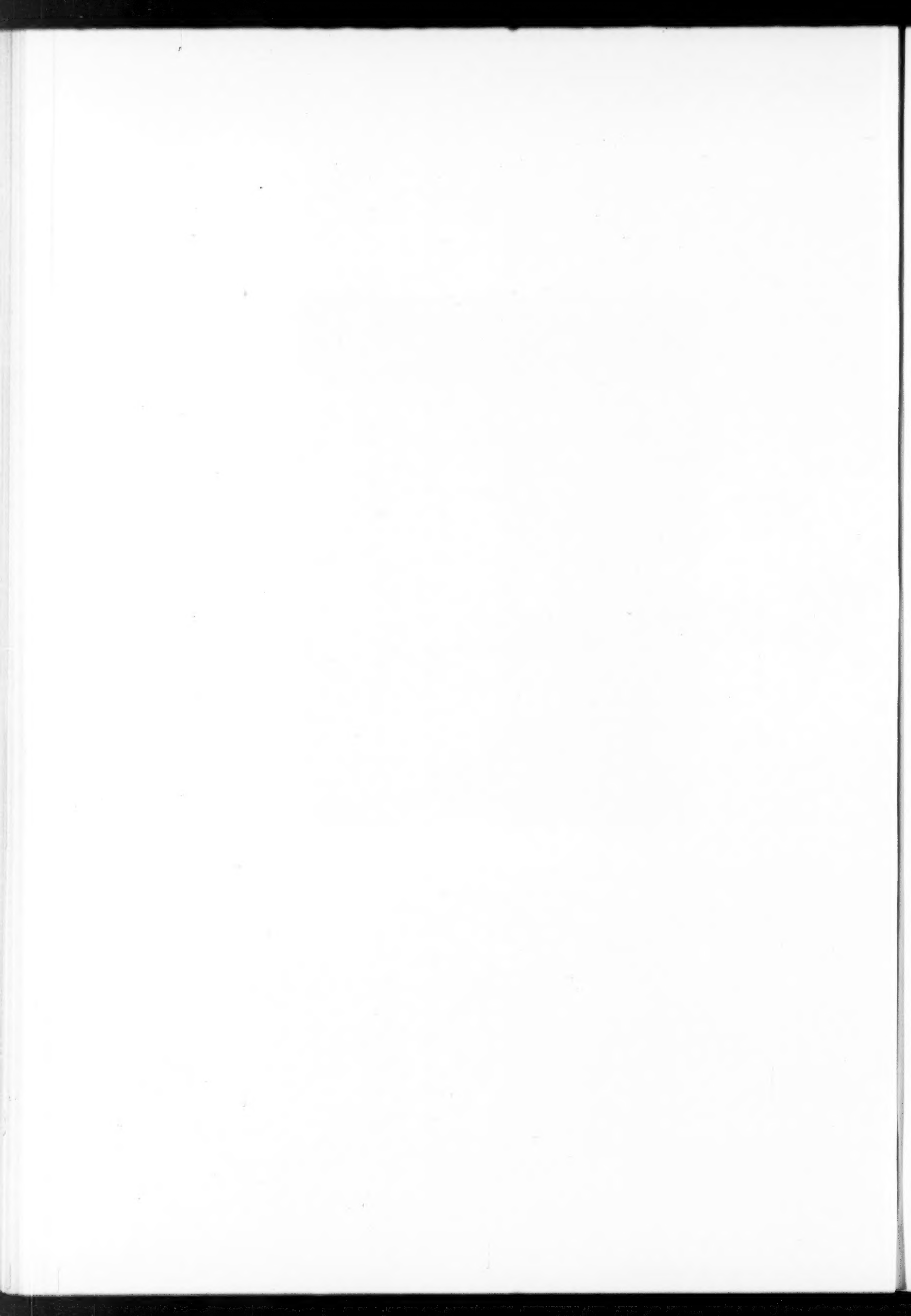
CASE D.—Male: aged 40. Had haematemesis two years ago with occasional diarrhoea; now suffers from dyspepsia. No fever. Liver contracted, very hard; spleen also very hard. Chronic type, advanced stage. Duration uncertain.







CASE E.—Male: aged 22. History of fever four months ago, followed by onset of ascites. Temperature slightly irregular. Abdomen full of fluid: 10.5 kilos removed. Liver felt cirrhotic; spleen not palpable. Advanced stage with ascites, marked emaciation.



# BIO-CHEMICAL AND THERAPEUTICAL STUDIES ON TRYPANOSOMIASIS

BY

ANTON BREINL AND M. NIERENSTEIN

*From the Runcorn Research Laboratories of the Liverpool School  
of Tropical Medicine*

*(Received for publication 15 August, 1909)*

CONTENTS.	PAGE
Introduction .....	396
Organic arsenic compounds .....	396
A. Atoxyl .....	396
B. Derivatives of Atoxyl .....	403
C. Orsudan .....	406
D. Derivatives of Orsudan .....	408
Colouring matters .....	409
Antimony compounds .....	412
The regularity of the time of re-appearance of parasites after treatment .....	417
Value of subinoculations.....	417
When can an animal be considered to be cured?.....	418
Comparative value of experiments on different laboratory animals	420

The following is a detailed report of our observations on experimental Trypanosomiasis, the treatment of infections with different pathogenic trypanosomes, and the mechanism of the therapeutical action of various trypanocidal compounds. The experiments were carried out in this laboratory during the last two years.

Our thanks are due to Sir Alfred Jones, through whose generosity we were able to extend our experiments upon monkeys. Further, we wish to express our indebtedness to Messrs. Burroughs, Wellcome and Co., who supplied us with a great number of trypanocidal drugs and supported the work very materially. We also have to express our thanks to Professor Ehrlich for the supply of different compounds prepared in his laboratory, and to Messrs. Bayer and Co. who put at our disposal a number of aniline dyes for our experimental work.

## INTRODUCTION

The ease with which infection caused by the different pathogenic trypanosomes can be transmitted from animal to animal, and especially the fatality of such infection in small laboratory animals, has attracted the attention of many workers.

The action of various compounds which are chemically allied has been studied with the object of finding an absolute cure for different trypanosome infections. This would obviously be of the utmost economic importance in different parts of the tropics.

Efforts in this direction have led to great progress in our knowledge of the biology of trypanosomes; they have thrown much light on protozoal diseases in general; they have advanced our experience in experimental chemotherapeutics, and have opened up a new and large field for original investigation.

The large number of drugs of specific trypanocidal character can be classified into three distinct groups. To the first group belong compounds containing as their active principle arsenic in an inorganic form, such as Sodium arsenate; to the second, those in which the arsenic molecule is masked by different organic radicals, such as the aniline nucleus, in Atoxyl and numerous allied compounds. To this second group also belong the different colouring matters of the diazo type: trypanred, trypanblue, and derivatives of the triphenylmethan type, such as malachite green, parafuchsin and trypanosan. The third group consists of Antimony in the form of sodium antimonyl tartrate and in the form of the three isomeric arylstibinic acids.

## ORGANIC ARSENIC COMPOUNDS

### A. *Atoxyl*.

Shortly after the discovery of the therapeutic value of Atoxyl in experimental Trypanosomiasis, this drug was extensively studied. It has been applied in the treatment of Sleeping Sickness and natural trypanosome infection in cattle, with very varying results. The mechanism of its action has been carefully studied, and Atoxyl may be regarded at present as one of the best known drugs.

Thomas and Breinl\* first introduced Atoxyl into the treatment of

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(b) Thomas and Breinl. *Liverpool School of Tropical Medicine, Memoir XVI*, 1905.



experimental Trypanosomiasis with a view to administering arsenic in larger doses and in a less toxic form than sodium arsenate, which was already known to possess a specific action on *T. brucei*.

Their work was continued by Moore, Nierenstein and Todd,\* who concluded that the specific action of Atoxyl on trypanosomes can not only be explained on the assumption of a slow breakdown of Atoxyl into an inorganic arsenical and aniline ion, but is also 'due to direct and specific action of a complex organic ion containing both the aniline and arsenical groups.'†

Mesnil and Brimont‡ explain the action of Atoxyl on trypanosomes in a similar way. Their conclusions are based on the observation of a specific resistance of trypanosomes to drugs of the same type, but not to those of different types, such as parafuchsin on the one hand and Atoxyl and acetylated Atoxyl on the other hand, a fact which proves 'que l'atoxyl n'agit pas uniquement comme arsenical, mais en vertu d'un ion complexe.'

Uhlenhuth and Woithe§ incline to this view, as the symptoms in experimental animals caused through toxic doses of Atoxyl, are not those of either aniline or arsenic poisoning. They 'beziehen diese ganz besonders gearteten nervösen Symptome auf den ganzen Atom-complex des Atoxyls.' The Atoxyl as such has a specific action on the cells of the organism, after they have undergone a change in some way or other under the influence of the parasites. This hypothetical action on the cells results in an increased formation of specific antibodies, which still continues even after the Atoxyl has been eliminated from the organism. These antibodies destroy the greater number of the parasites and prevent the few surviving trypanosomes from multiplying and injuring the host.¶

\* (a) Moore, Nierenstein and Todd. Bio-chemical Journal, Vol. II, No. 4-5, 1907.

(b) Moore, Nierenstein and Todd. Annals of Tropical Medicine and Parasitology, Vol. II, No. 4, 1909.

† Moore, Nierenstein and Todd. Bio-chemical Journal, Vol. II, No. 4-5, p. 322, 1907.

‡ Mesnil and Brimont, Comptes Rendus de la Soc. de Biol., Tome LXIV, 1908, p. 639.

§ Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Band XXIX, 1908. Reprint p. 34.

¶ Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Bd. XXVII, 2. Heft, 1907.

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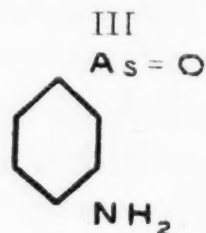
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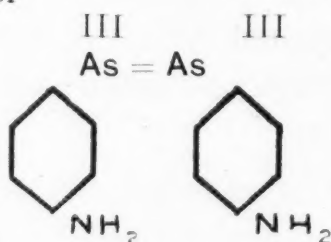
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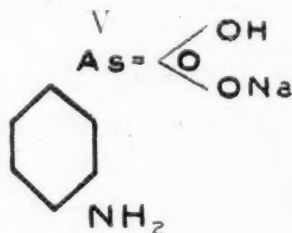
On the other hand, Ehrlich\* and his collaborators express quite a different view of the action of Atoxyl. They assume that Atoxyl changes in the organism into a compound of a more toxic, and, therefore, more trypanocidal character, and consider that the pentavalent arsenic atom in Atoxyl becomes reduced in the organism to a trivalent atom, which form they regard as the active trypanocidal arsenic. Their view is based on the observation that p-amino-phenyl-arsen-oxide



and di-amino-arseno-benzol



(both reduction products of Atoxyl) have a marked trypanocidal action *in vitro*, whereas Atoxyl



as has been shown by a great number of observers, does not influence the parasites *in vitro*.

They believe, therefore, that the Atoxyl molecule is reduced in the organism, and products of a similar type, such as p-amino-phenyl-arsen-oxide and di-amino-arseno-benzol, are formed. Röhl,† Ehrlich's co-worker, goes so far as to state that the action of Atoxyl is only due to the formation of p-amino-phenyl-arsen-oxide.

\* (a) Ehrlich Verhandlungen der deutschen dermatologischen Gesellschaft, X. Congress, 1908.

(b) Ehrlich. Berichte der deutschen chemischen Gesellschaft, Jg. XLII, Heft I, 1909.

† Röhl. Berl. klin. Wochenschrift, No. 11, 1909.



They assume in the protoplasm of the trypanosome the existence of a special chemical complex (Arseno-receptor) which possesses a great affinity for trivalent arsenic.

Ehrlich's theory led to Levaditi's and Yamanouchi's\* studies on the mechanism of the action of Atoxyl. By the action of an emulsion of animal tissue, especially liver, they were able to transform Atoxyl, which is inactive *in vitro*, into a trypanocidal compound 'trypanotoxyl,' which they regard as a combination of reduced Atoxyl with protein, a 'toxalbumine arsénée' of a thermolabile character. Levaditi compares this 'toxalbumine arsénée' with a haemolysin; the arsenic plays the part of a complement; the protein nucleus, the rôle of an amboceptor. The arsenic, therefore, cannot attach itself to the trypanosomes without the inter-currence of the protein nucleus of the toxalbumine arsénée.†

In a somewhat different way Friedberger‡ succeeded in transforming Atoxyl into a trypanocidal compound *in vitro* by adding thioglycolic acid to a solution of Atoxyl. The leading idea in his experiments was Heffter's theory of the reductive properties of the organism; according to the latter the protein 'reductases' are not to be considered living ferments, but the reduction is brought about by sulphhydryl or SH groups which also are present in this glycolic acid.

Our experiments,§ however, have brought forward very little evidence for the supposition that a reduction process in the organism plays a prominent rôle in the action of Atoxyl on trypanosomes. As a matter of fact, all our results tend to show that the transformation of Atoxyl into a powerful trypanocide *in vitro* and *in vivo* is mainly

\* (a) Levaditi et Yamanouchi. Comptes Rendus de la Soc. de Biol. Tome LXV, p. 23, 1908.

(b) Levaditi, Brimont et Yamanouchi. Comptes Rendus de la Soc. de Biol., Tome LXV, p. 25, 1908.

(c) Levaditi. Bull. de la Soc. de Path. exotique, Tome II, p. 45, 1909.

(d) Levaditi. Comptes Rendus de la Soc. de Biol., Tome LXVI, p. 33, 1909.

(e) Levaditi. Comptes Rendus de la Soc. de Biol., Tome LXVI, p. 492, 1909.

† Röhl in a more recent publication (Zeitschrift f. Immunitätsforschung, Vol. II, p. 496, 1909), points out that Levaditi does not furnish any conclusive proof for his conception of the formation of trypanotoxyl in the organism, and asserts that the trypanocidal effect of Atoxyl *in vivo* is only due to the formation of p-amino-phenyl arsenoxide.

‡ Friedberger. Berl. klin. Wochenschrift. No. 38, 1908.

§ Breinl and Nierenstein. Zeit. f. Immunitätsforschung, etc., Vol. I, p. 620.

due to an oxidation process. A reduction takes place in the intestines only, and is probably of secondary importance.

In our experiments we adopted Levaditi's and Yamanouchi's technique; after repeated failures to get any changes in an Atoxyl solution when shaken up for twenty-four hours at room temperature with an emulsion of different organs (liver, brain, etc.), 10 c.c. of a 4 per cent., 2 per cent., and 0.2 per cent. solution of Atoxyl in physiological saline were mixed with an equal amount of liver emulsion and kept at a temperature of 37° C. After two hours the effect of this mixture on trypanosomes was tried in a coverslip preparation. Only three experiments out of seven confirmed Levaditi's and Yamanouchi's observation that the parasites became immobilised and after a time destroyed.

After dialysing an Atoxyl-liver mixture which had been found to have a marked trypanocidal action in a coverslip preparation and concentrating the dialysate to the original volume, when it became again isotonic, we were able to observe that the dialysate acted upon the parasites in the same way as the original Atoxyl-liver mixture; the slightly acidified dialysate gave a distinct precipitate of arsenic sulphide when acted upon by  $H_2S$ .

*We found that only in those cases in which, on mixing Atoxyl solution with liver emulsion, inorganic arsenic was present had the mixture a decided and rapid trypanocidal effect in vitro. On the other hand, in no case in which arsenic could not be detected, could any influence on the parasites be observed.*

Similar results with regard to the appearance in the solution of inorganic arsenic were obtained when pure liver oxydase or oxydase of black tea was added in a sufficient quantity to 1 per cent. solution of Atoxyl, or when  $H_2O_2$  was mixed with a 4 per cent. or 2 per cent. solution of Atoxyl and exposed for some time to a temperature of 37° C.

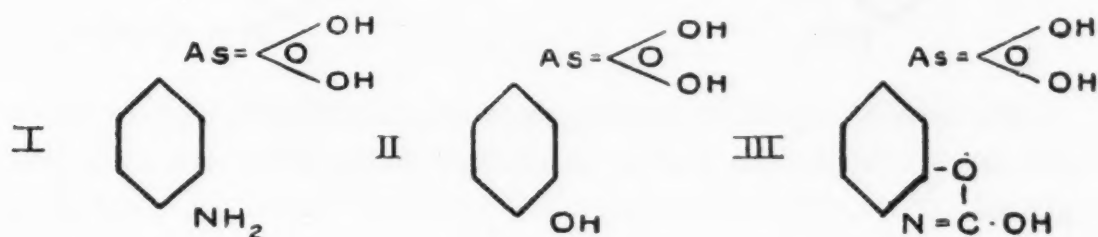
If reductase prepared from yeast was added to Atoxyl solutions of different strengths, in some instances inorganic arsenic was set free from the Atoxyl together with aniline. This observation offered an explanation of the presence of aniline in the faeces, previously recorded.\*

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\* Nierenstein. *Annals of Tropical Medicine and Parasitology*, Vol. II, No. 4, 1909.

In order to imitate as far as possible, *in vitro*, the changes which Atoxyl undergoes in the organism, an 'Atoxyl-serum' was prepared by mixing a solution of Atoxyl with serum and dialysing out the excess of Atoxyl which had not combined with the serum proteins through the amino group. On the addition of liver emulsion or  $\text{H}_2\text{O}_2$  to this 'Atoxyl-serum' inorganic arsenic was set free.

Nierenstein's\* research on the mode of elimination of Atoxyl in the urine confirms our conception that Atoxyl mainly undergoes oxidation in the organism. After injecting the drug into a horse, it was recovered in the urine in the form of p-amino-phenyl-arsenic acid (Formula I), p-oxy-phenyl-arsenic acid (Formula II), and oxy-carb-amino-phenyl arsenic acid (Formula III).



It is obvious that the formation of the compounds II and III from I (the mother substance of Atoxyl) can only be explained through an oxidation process in the organism.

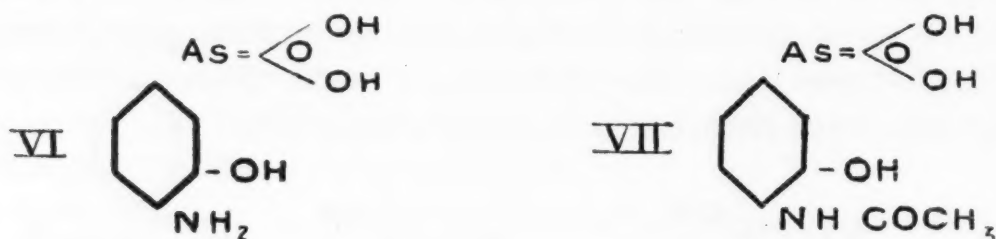
The formation of p-oxy-phenyl-arsenic acid (Formula II) from p-amino-phenyl-arsenic acid is brought about through a replacement of the amino group by an HO group, and is similar to the formation of p-amino-phenol (Formula IV) after injection of p-amino-aniline (Formula V).



This oxidation process enables the organism to eliminate aniline and Atoxyl in a less toxic form as the sulphuric or glycuronic derivatives.

\* Nierenstein. Zeit. f. Immunitätsforschung, Bd. II, No. 4, 1909.

The formation of oxy-carb-amino-phenyl-arsenic acid can be explained in the following way. The organism introduces on oxidation an OH group in the o-position to the amido group, and thus forms p-amino m-oxy-phenyl-arsenic acid (Formula VI). This oxidation is then followed (based on the analogy of toluidine) by an intermediate acetylation of p-amino- m-oxy-phenyl-arsenic acid in the organism (Formula VII). The acetyl chain is oxidized, and forms oxy-carbonyl arsenic acid (Formula III).



On considering the foregoing experiments and results, it becomes possible to divide the action of Atoxyl in the organism into four phases.

I. After injection of Atoxyl, a comparatively small amount combines through the amino group with the serum proteins and forms 'Atoxyl-serum'; the greater part is secreted in the urine, partly unchanged as p-amino-phenyl-arsenic acid, partly oxidised in the form of p-oxy-phenyl-arsenic acid and oxycarb-amino-phenyl-arsenic acid, and partly as free inorganic arsenic.

II. From the Atoxyl serum, arsenic is set free through an oxidation process, caused by the oxidative ferments present, and probably also by the trypanosomes, whereby the aromatic nucleus is destroyed.

III. At the same time a reduction process takes place in the intestines, whereby the Atoxyl molecule is reduced to aniline and arsenious acid.

IV. The arsenic which is formed through oxidation acts *in statu nascendi* on the trypanosomes.

As has been pointed out before, the action of Atoxyl can be compared to that of a dye.\* The amino group plays the rôle of a

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\* Nierenstein. Annals of Tropical Medicine and Parasitology, Vol. II, No. 3, p. 249, 1908.

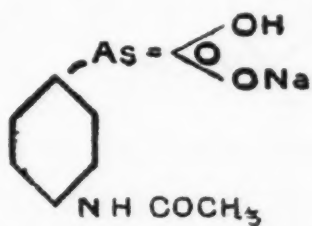


chromogenetic group, the arsenical radical acts on the parasites in the same way as a chromophoric group on a cotton fibre, whereas the serum plays the rôle of a mordant.

## B. Derivatives of Atoxyl.

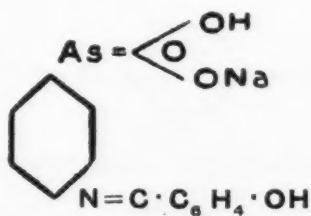
In order to ascertain whether certain derivatives of Atoxyl have a superior therapeutical value in trypanosome infections, a great number of compounds were experimented with.

### 1. Acetylated Atoxyl.



Acetylated Atoxyl was first prepared by Ehrlich and Bertheim. It was used by C. Browning, by Moore, Nierenstein and Todd, and by Breinl. It proved to be less toxic for animals very susceptible to Atoxyl, such as dogs, which, as Ehrlich\* points out, is not the case in horses and guinea-pigs. The only difference between it and Atoxyl, consists in the partial acetylation of the amino group, which change apparently reduces the toxic effect of the aniline nucleus in the Atoxyl. Uhlenhuth and Woithe† believe that the acetyl group prevents the rapid discharge of arsenic, a conception which is improbable. It has been pointed out by Nierenstein, that part of Atoxyl undergoes acetylation in the organism, which observation probably explains the less toxic effect of acetylated Atoxyl.

### 2. Salicyli-Atoxyl.



This compound was prepared with the view of introducing Atoxyl *in statu nascendi* into the organism, as it might be expected that on

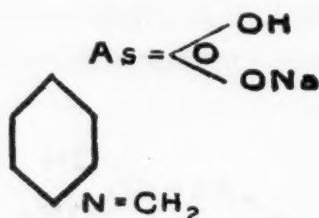
\* Ehrlich. Berl. klin. Wochenschrift, No. 9-12, 1907. Reprint, p. 22.

† Uhlenhuth und Woithe. Arb. aus dem Kaisl. Gesundheitsamte, Band XXIX, 1908. Reprint, page 42.

hydrolysis, p-amino-phenyl-arsenic acid and salicylic acid would be formed. The salicylic acid might then act as an internal disinfectant.

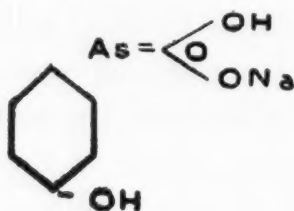
A few experiments on rats infected with *T. brucei* showed that doses of as much as 0.025 gm. per rat of about 170 gm. did not cause a disappearance of the parasites from the blood.

3. Formylo-Atoxyl.



Formylo-Atoxyl brought about in rats infected with *T. evansi* a temporary disappearance of the parasites after two to three injections of 0.025 gm.; the trypanosome, however, reappeared in the course of a few days. The drug caused abscesses at the site of injection.

4. Sodium p-hydroxy-phenyl-arsenate.

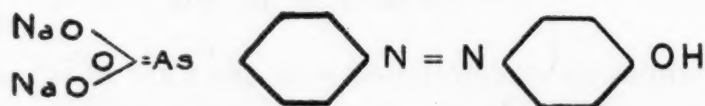


This compound was found to have no effect whatever on the parasites, a fact which corresponds with the view that the presence of the amino group is essential for a trypanocidal action.

5. Di-sodium Azobenzene 4-arsenate



and Di-sodium 4-oxy Azobenzene 4-arsenate.



From a theoretical point of view, it might be expected that after injection both compounds would on reduction in the organism break

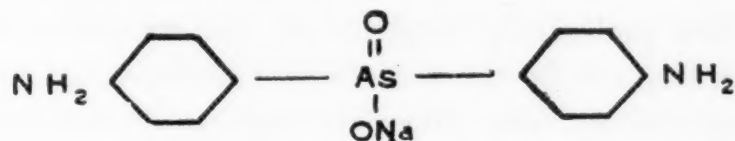
up into free p-amino-phenyl-arsenic acid, and that the latter, together with an amino group *in statu nascendi*, might have a greater affinity for the serum proteins and combine with them to a larger extent. Experiments, however, have shown that this process does not take place in the organism, as even large doses had no appreciable effect on rats infected with trypanosomes.

6. Tetra sodium phenazine 4-arsenate.

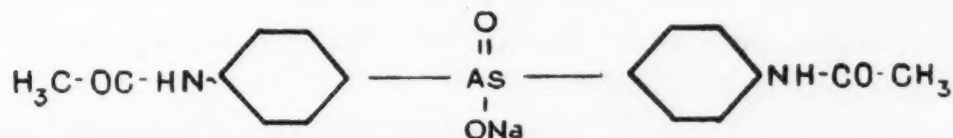


The destructive effect of phosphine, a colouring matter of the safranine type,\* on *Paramaccium* is very marked, and is not surpassed by any other substance. We, therefore, hoped that on combining an arsenic molecule with a phenacine, a colouring matter of the same type, the result would be a compound of increased trypanocidal value in comparison with Atoxyl. Experiments, however, proved that this compound did not affect trypanosomes. Reference may here be made to the work of Mannaberg,† who finds that phosphines have no beneficial effect in the treatment of Malaria.

7. Sodium di-p-amino-phenyl-arsenate



and Sodium di-p-acetyl-amino-phenyl-arsenate.



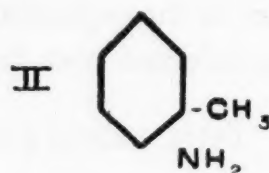
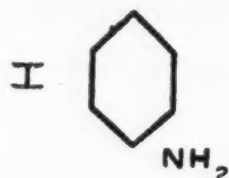
These compounds, even if used in the largest doses which could be administered, were of no value in the treatment of rats infected with *T. equiperdum*.

\* Frankel M. Die Arzneimittelsynthese auf Grundlage der Beziehg. zwischen chemischen Aufbau und Wirkung. 2. Aufl., 1906, p. 206.

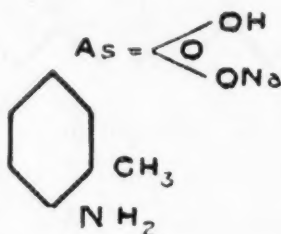
† Mannaberg. Arch. f. klin. Medizin, Bd. 59, p. 185.

C. *Orsudan*

Experiments on a larger scale have been carried out with substances closely allied to Atoxyl, but having, instead of an aniline nucleus (Formula I), a toluidine nucleus (Formula II).

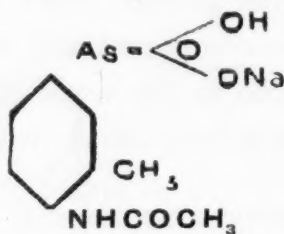


## I. Sodium 3-methyl 4-amino-phenyl-arsenate (Kharsin)



and its acetyl derivative.

## Sodium 3-methyl 4-acetyl-amino-phenyl-arsenate (Orsudan).



After a few preliminary experiments the acetylated compound Orsudan was found to be the less toxic of the two, and most of the treatment experiments were carried out with it. It was a remarkable fact that Orsudan did not have any effect on rats or on one donkey infected with *T. brucei*, although two strains of this parasite, of different origin, were used. On the other hand, it acted promptly on *T. equiperdum* and *T. gambiense*.

In rats infected with *T. equiperdum*, parasites disappeared after the first injection of 0.025 gm. of the drug, but usually recurrences were observed after a varying period if treatment was discontinued. Only a few rats lived as long as eight months without reappearance of parasites, but these also succumbed to intercurrent diseases. Two rats showed relapses after a very prolonged period, one, 126 days after the last injection of Orsudan (0.075 gm. had been administered),



the other, 105 days after discontinuance of treatment (0.06 gm. of Orsudan was administered in three injections).

Out of twelve rats infected with *T. gambiense* one lived for 165 days, one for 115 days (both dying without having shown parasites in the blood), and one had a relapse fifty days after the last injection.

Guinea-pigs infected with *T. gambiense* stood Orsudan much better than Atoxyl. The parasites always disappeared from the blood after the first injection of 0.025 gm. When the treatment was discontinued, relapses set in after a varying interval. If treatment was continued the intervals between the relapses became shorter and shorter, until a point was reached when even repeated injections had no effect on the parasites, and the animal succumbed to the trypanosome infection. If the parasites were subinoculated into guinea-pigs, one injection of Orsudan was sufficient to drive out the parasites.

Three donkeys, infected with *T. equiperdum*, were treated with Orsudan. Two died after two injections (each of one gramme) after typical symptoms of arsenic poisoning lasting three days. The internal organs showed fatty degeneration of the liver and kidneys. One donkey died of Trypanosomiasis 227 days later. It received, during this period, nine injections, each of one gramme of Orsudan; parasites in small numbers were occasionally seen in the blood.

Four monkeys infected with *T. gambiense*, after the infection was well established, were treated with Orsudan. One *Cercopithecus callitrichus* died with all the symptoms of typical arsenic poisoning, after having received 0.2 gm. in two injections. One *Cercopithecus mona* received 0.2 gm. in two injections, and had a relapse forty-nine days after the last injection. The treatment was repeated with 0.1 gm. of Orsudan. Although symptoms of arsenic poisoning set in a few days afterwards, they passed off in a week, and the animal lived for 254 days in good health without showing any parasites. On reinoculation of *T. gambiense* it succumbed to the infection.

One *Cercopithecus callitrichus* received 0.2 gm. of Orsudan in two injections. Three days afterwards severe symptoms of arsenic poisoning set in, which, however, passed off in a week's time. Twenty-five days afterwards treatment was resumed with one injection of 0.1 gm. Orsudan. Two days afterwards symptoms of arsenic poisoning commenced; four days later failure of eyesight was noticed,

and within three days the animal was totally blind. As no recovery of vision took place, the animal was killed with the intention of examining the pathological lesions of the eye.

One *Macacus rhesus* was inoculated with *T. gambiense*. Treatment was begun after the second natural relapse. After one injection of 0.1 gm. of Orsudan the parasites promptly disappeared. Thirty-five days afterwards a second injection of 0.1 gm. of Orsudan was given. During the following day symptoms of severe arsenic poisoning set in, and the animal became totally blind six days after the injection. This monkey is still alive thirteen months after the inoculation. He is absolutely blind, and no visible changes can be noticed in his eyes.

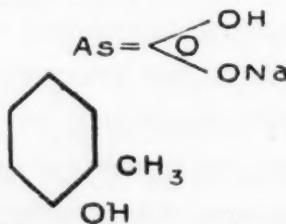
The foregoing experiments lead to the conclusion that Orsudan as a trypanocide is, for *T. gambiense* and *T. equiperdum* in experimental animals, nearly equal to that of Atoxyl.

As with Atoxyl, only in a very small percentage of our experiments have we been able to prevent relapses. On the other hand, Orsudan is certainly very toxic indeed, as our experiments in monkeys show; out of four animals treated, two cases of blindness and one death from typical arsenic poisoning occurred. The higher toxicity, when compared with Atoxyl, may be explained on the hypothesis that Orsudan combines to a larger extent with the tissue than Atoxyl, owing to the fact that  $\text{CH}_3$  group is oxidised to  $\text{COOH}$ , and, therefore, more arsenic combines and is afterwards set free in the organism.\*

In a few of our experiments on rats, haemoglobinuria, the result of haemolysis, was noticed. This may be due to the well-known fact that toluidine derivatives are more powerful haemolytic agents than aniline derivatives.

#### D. Derivatives of Orsudan.

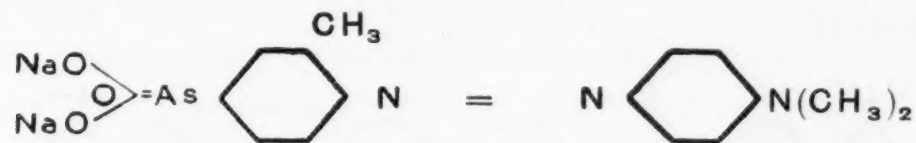
##### 1. Sodium 3-methyl 4-hydroxy-phenyl-arsenate.



\* Lately we have had an opportunity of testing a new sample of Orsudan. Two successive injections of 0.2 gr. into a monkey did not give rise to any symptoms of arsenical poisoning.

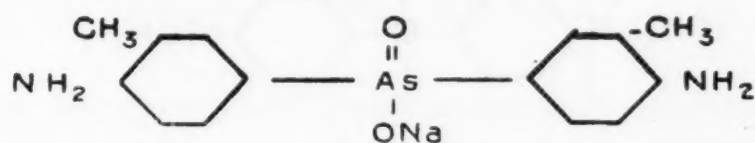
The replacement of the amino group by an hydroxyl group changes the character of the compound in the case of Orsudan as it does in Atoxyl. It is extremely toxic and showed only a very slight action on the parasites, and only when injected in so large doses as 0.05 gm., in which case it killed the animal in a few hours.

2. Di-sodium-4-di-methyl-amino 2-methyl-azo-benzene 4-arsenate.

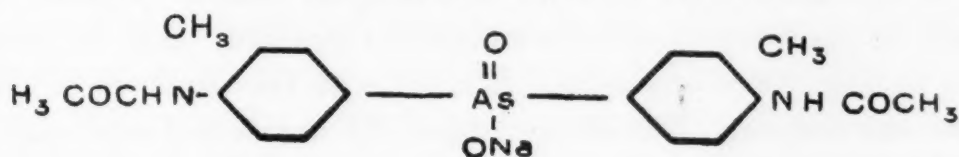


was found to have a slight effect on trypanosomes. They decreased in number, but the rats usually died within a few days. When compared with the corresponding Atoxyl derivative No. 5, p. 401, it is seen that although the combining amino group is saturated through diazotising, in this case the  $\text{CH}_3$  group most probably gets oxidised to a  $\text{COOH}$  group, through which a combination with the proteids takes place.

3. Sodium di-(3-methyl 4-amino-phenyl) arsenate



and Sodium di-(3-methyl 4-acetyl-amino-phenyl) arsenate

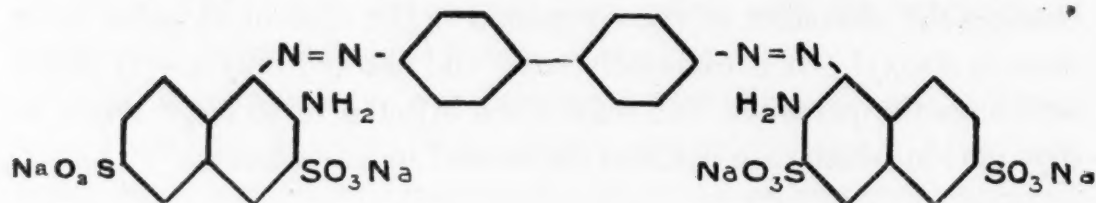


had, in the same way as the corresponding Atoxyl derivative, on injection, no effect on rats infected with *T. equiperdum*.

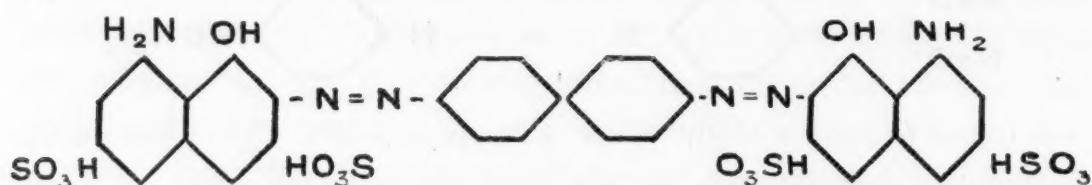
### COLOURING MATTERS

Since Ehrlich and Shiga's discovery that trypanred has a pronounced trypanocidal action, numerous colouring matters of different chemical constitution have been tried in experimental Trypanosomiasis with a more or less therapeutical success.

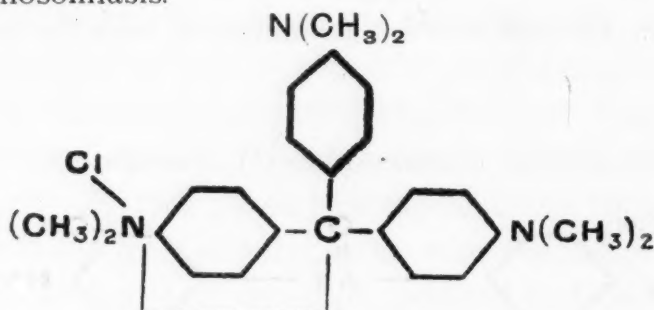
The preparation of trypanred by Ehrlich and Shiga (Formula I)



has led to the discovery of afridol blue by Nicolle and Mesnil (Formula II).



Afterwards Ehrlich introduced parafuchsin (Formula III) and trypanosan, a chlorinated parafuchsin, in the treatment of experimental trypanosomiasis.



The value of all these colouring matters has been put to a severe test in this laboratory, but with discouraging results. Thomas and Breinl\* in 1905 remarked in reference to trypanred, 'that we cannot claim to have cured any animal infected with the parasites of surra, ngana and dourine. The disease, especially in rats and mice, may be greatly prolonged, but the animals eventually die.' Moore, Nierenstein and Todd† came to a similar conclusion 'that trypanroth was not always able to prevent an early death from the disease.'

Afridol blue and parafuchsin, phosphines and numerous other colouring matters, had in our hands only a slight effect, or no effect at all, on the parasites. We may say that hardly any work with these

\* Thomas and Breinl. Liverpool School of Tropical Medicine, Memoir XVI, p. 51, 1905.

† Moore, Nierenstein and Todd. Annals of Trop. Med. and Parasitol., Vol. II, No. 4, p. 285.



colouring matters was carried out on mice, a fact which may account for our inability to confirm Ehrlich's experiments.

Ehrlich\* suggested that parafochsin may act as a prophylactic for trypanosome infection. He was successful in preventing infection in mice after feeding them with parafochsin and then inoculating the parasites.

We attempted to confirm these experiments on large animals. Two large horses were fed on parafochsin. One of them received fifteen grammes daily by mouth, for thirty days and died after having shown toxic symptoms from the parafochsin. A second horse received fifteen grammes by mouth for forty-eight days. On inoculation, it became infected in the same way as an untreated animal.

In our opinion, it is not necessary to compare the action of colouring matters on trypanosomes with the action of Atoxyl. Whereas in Atoxyl the amino group, according to our experience, effects a combination between the proteins and the Atoxyl molecule, and the specific action on the parasites is due only to the liberated arsenic; in colouring matters the trypanocidal effect is most probably due to the amino group. Mesnil and Nicolle† already expressed the view that the therapeutical effect may be due to the presence of nitrogen.

Moore, Nierenstein and Todd‡ came to the conclusion that the  $\text{NH}_2$  group is the active trypanocidal radical, for which they suggest the name 'trypanophobe group.' This conception corresponds to the observations of Loew and Bokorny§ on the influence of compounds containing amino groups upon the multiplication of algae. They found that with an increase of the number of amino groups in compounds of the type of urea and uric acid, the noxious influence upon the plant increases. They explain the observation in this way: that the protoplasm contains a great number of labile aldehyde and amino groups which combine alternately with the amino and aldehyde

\* Ehrlich. Berl. klin. Wochenschrift, 1907. Reprint, p. 31.

† Mesnil and Nicolle. Annales de l'Institut. Pasteur, XX, p. 417, 513, 1906.

‡ Moore, Nierenstein and Todd. Annals of Tropical Medicine and Parasitology, Vol. II, No. 4, 1909, p. 271.

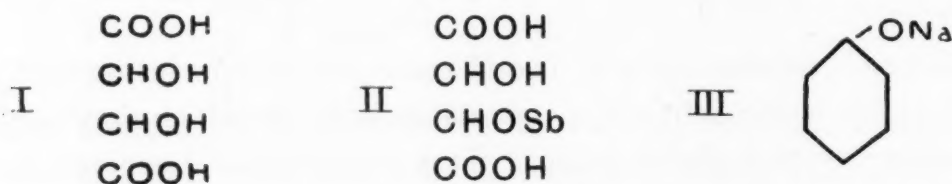
§ Loew und Bokorny. Jour. f. prakt. Chemie, Bd. 36, p. 272. (Compare, Fränkel, *loc. cit.*, p. 29.)

groups of the drug. This theory may be applied to the action of aniline dyes upon trypanosomes. In this case the labile aldehyde groups of the protoplasm of the trypanosome may combine with the amino group of the drug. Gabritschewsky\* explains in a similar way the slight neutralising action of fuchsin and vesuvin if brought in contact with toxins.

### ANTIMONY COMPOUNDS

Plimmer and Thompson† were the first to introduce antimony in the treatment of experimental Trypanosomiasis. Their results were encouraging. These good results, however, have not been fully confirmed by other workers. Plimmer and Thompson used sodium, potassium and lithium salts of antimonyl tartrate. On considering the chemical constitution of these compounds, it is apparent that they were not, in fact, dealing with an organic compound of Antimony, but only with an alcoholate of tartaric acid (Formula I) and antimonite oxide.

Antimonyl tartrate has the following constitution (Formula II). The antimonite oxide in this case is not in an aromatic chain as the arsenic in Atoxyl, but forms only an alcoholic salt similar to sodium in a phenolate (Formula III).



The action of sodium antimonyl tartrate is an antimonite action pure and simple. On injection, dissociation of the compound into sodium, tartaric acid, and antimonite oxide takes place, the latter of which acts destructively on the parasites. A similar process takes place on mixing the compound with infected blood, even in the dilution of 1 : 40,000 the trypanosomes are immobilized in a few minutes.‡

\* Gabritschewsky. Arch. Internationales de Pharmacodynamie et de Therapie, Vol. VII, Fasc. 1-2, p. 115.

† Plimmer and Thompson. Proc. of the Roy. Soc. B., Vol. 80, 1907.

‡ Broden et Rodhain. Travaux du laboratoire médical de Leopoldville, III, 1909, p. 48.

This rapid liberation of antimonie oxide, which, as is well known, is very slowly resorbed by the tissues and causes great irritation, may be the cause of abscess formation at the site of injection. This disadvantage of abscess formation was overcome by introducing an organic antimony compound of a similar type to Atoxyl into the therapeutics of Trypanosomiasis,\* the antimony radical being linked on to the aromatic nucleus. From the three isomeric arylstibinic acids only the para derivative gave satisfactory results. The meta compound was far less stable, as it partly decomposed on standing into antimonie oxide and aniline. The ease with which this compound decomposes may be held responsible for the occurrence of abscesses after injection. The antimony is probably too quickly liberated and not sufficiently quickly resorbed.

The mechanism of the action of the p-amino-phenyl-stibinic acid is similar to that of p-amino-phenyl-arsenic acid (Atoxyl). It is remarkable on comparing the action of sodium antimonyl tartrate and of sodium p-amino-phenyl-stibinic acid, that whereas the first named compound effects a disappearance of the parasites within one hour, the latter compound produces the same effect only after the lapse of 15-19 hours. The effect stands in direct proportion to the rate at which antimony is set free from the drugs.

### RESISTANCE

Thomas and Breinl† observed in their work on Atoxyl in experimental trypanosomiasis, that in a horse infected with *T. evansi*, the parasites disappeared from the blood after administration of Atoxyl. In spite of continuation of the treatment, parasites were seen again, and 'were twenty-five to a field on the fourth day after the dose in the last week but one. Two doses per week were therefore commenced; this kept the number of parasites down, so that one to five fields was the highest reached.'

Similar observations were made by Mesnil and Nicolle,‡ using benzidine colours for the treatment of experimental trypanosomiasis.

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\* Breinl and Nierenstein. *Annals of Tropical Medicine and Parasitology*, Vol. II, No. 5, 1909.

† Thomas and Breinl. *Liverpool School of Tropical Medicine, Memoir XVI*, 1905, p. 65.

‡ Mesnil and Nicolle. *Annales de l'Institut. Pasteur*, No. XX, p. 528, 1906.

They found that after continuation of the treatment for some time the drug had no effect upon the parasites.

Franke\* found, 'working with mice infected with Ngana and treated with parafuchsin, that when the recurrences ceased to respond to the treatment, inoculation into fresh animals gave rise to an infection which was from the very beginning uninfluenced by parafuchsin, administered either by feeding or by injection.'

Browning† then observed a similar behaviour of certain trypanosome strains against Atoxyl.

It was, however, Ehrlich who first recognised the general importance of this phenomenon, and drew attention to the fact that one can produce strains resistant to all types of trypanocides. He had at his disposal strains resistant against—(1) Atoxyl, (2) Trypan-red, (3) Trypanblue, (4) Atoxyl and trypanblue, (5) Arseno-phenyl-glycine, (6) Tartar emetic.‡

Ehrlich was further able to prove that this resistance may be preserved throughout many passages in mice, and he regards this feature as 'einen schönen Beweis für die Vererbung erworbener Eigenschaft.' However, in the case of one strain (Atoxyl strain, No. 1), which kept resistant for six months (67 passages), this acquired character was lost after seven and three-quarter months (87 passages). A striking characteristic of the resistance is its specific nature, i.e., if a strain has become resistant to parafuchsin it is resistant as well against all colouring matters of the tri-phenyl-methan

\* Quoted by C. H. Browning. *Journal of Path. and Bacteriology*, Vol. XII, 1908, p. 176.

† Ehrlich. *Berl. klin. Wochenschrift*, No. 9-12, 1907. Reprint, p. 22.

‡ Strains of trypanosomes resistant to tartar emetic have also been obtained by Mesnil and Brimont, by Plimmer and Bateman; and a strain of *T. brucei* resistant against p-amino-phenyl-stibinic acid has been obtained by us. This resistance, however, was not transmissible on subinoculation. Mesnil and Brimont succeeded in obtaining a permanent antimony resistant strain by treating an Atoxyl-resistant strain with antimony. At first the parasites were influenced by tartar emetic, but after the fifth injection they became resistant, a resistance which persisted for seventy-six passages.

A similar relative resistance was observed by us when treating guinea-pigs infected with *T. gambiense* with Orsudan. After six to seven injections the drug had lost its influence on the parasites.

Uhlenhuth, Hübner and Woithe, working with *T. equiperdum*, were not able to confirm entirely Ehrlich's observations. They were only able to produce a resistance in one animal after a certain number of relapses. On subinoculation, Atoxyl influenced the parasites in a normal way. Therefore, they regard it as a relative resistance, distinguishing it from an absolute resistance in Ehrlich's sense of the term.



group, though not resistant against Diazo-colouring matters, as trypanred, and arsenic compounds and *vice versâ*. Mesnil's observation contradicts to a certain extent this statement, as a strain resistant against Atoxyl was found to be influenced by Orsudan.

Ehrlich\* explains this complex phenomenon on the assumption that the avidity of the arseno-receptors of the trypanosomes for arsenyl has diminished to such an extent, that the protoplasm of the trypanosomes has become unable to combine with the Atoxyl.

The fact that an Atoxyl-resistant strain is still influenced by arseno-phenyl-glycine seemingly contradicts his conception. The interpretation which Ehrlich gives, is that although the trypanosomes of an Atoxyl-resistant strain have lost the greater part of the avidity of their arseno-receptors for Atoxyl, yet enough still remains to permit of the action of arseno-phenyl-glycine being manifested.

In the hands of one of us an Atoxyl-resistant strain in mice, sent by Professor Ehrlich† early in 1907, was shown to lose its resistance against Atoxyl when inoculated into rats.

Independently, Mesnil and Brimont‡ came to a similar conclusion, 'que la race est resistente à l'Atoxyl dans un organisme donné.'

Breinl and Nierenstein,§ in their work with an Atoxyl-resistant strain of *T. brucei* in donkeys, were able to prove that the acquired resistance against Atoxyl only holds good for a given species of animal, and that this acquired character is retained for this given species even after prolonged passages through different animals in which parasites are not resistant. On subinoculation of the Atoxyl-resistant parasites into rats, only in the first generation was a slight resistance noticeable. In the second generation the parasites behaved in the same way as the normal control strain. Mesnil and Brimont¶ find that an Atoxyl-resistant strain in mice is still influenced to a certain degree by Asodyl, very slightly by trisulphide of arsenic. Whether arsenious acid still effects Atoxyl-resistant trypanosomes or

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\* Ehrlich. Verhandlungen der deutschen dermatologischen Gesellschaft, X, Congress, 1908.

† Ehrlich. Jour. of the Roy. Inst. of Public Health, Vol. XX, No. 7, p. 391.

‡ Mesnil et Brimont. Comptes Rendus de la Soc. de Biol., Tome LXIV, 1908, p. 637.

§ Breinl and Nierenstein. Deutsche med. Wochenschrift, No. 27, 1908.

¶ Mesnil et Brimont. Annales de l'Institut Pasteur, Tome XXII, p. 856, 1908.

not, they were unable to decide definitely. They confirm the above-mentioned observations of Breinl and Nierenstein, laying particular stress on the point that a slight resistance may still be noticed when Atoxyl-resistant trypanosomes are subinoculated into a different species, such as from mice into a dog or a guinea-pig.

Röhl\* states that trypanosomes which are Atoxyl-resistant in mice are, on inoculation into rats, not absolutely resistant, though certainly so to some extent. He, however, does not explain whether this resistance was only noticeable in the first generation. Furthermore, he brings forward experimental evidence which tends to show that an Atoxyl-resistant strain in mice on subinoculation into rats is not affected by Atoxyl. However, as inoculation and injection were done simultaneously, in our opinion no definite conclusion can be formed.

We regard the resistance as an acquired immunity of the trypanosomes against the Atoxyl-serum, as we have previously pointed out. This is in accordance with our observation, confirmed by different observers, that the Atoxyl resistance only holds good for the one species in which it has been acquired. The trypanosomes have become tolerant only to the *one* Atoxyl-serum combination, as for example in the mouse, and are still influenced by the Atoxyl-serum of the rat.

A similar view has been expressed by Mesnil and Brimont.† In further experiments, however, with an Atoxyl-resistant strain in mice, sent to us by Professor Ehrlich, we observed that the resistance after subinoculation from mice into rats was still well marked in the latter animals even after three passages. This result, also obtained by Röhl, seemingly contradicts our previous observations that the resistance is confined to one animal species *only*. We agree with Röhl's opinion that the seemingly contradictory results concerning resistance can only be explained through the assumption of a gradual increase of the resistance, after numerous passages from a single animal, at first to animals of the same species, and later to animals of a different species.

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\* Röhl. Berl. Klin. Wochenschrift, No. 11, 1909.

† Mesnil et Brimont. Annales de l'Institut. Pasteur, Tome XXII, p. 856, 1908.

### THE REGULARITY OF THE TIME OF REAPPEARANCE OF PARASITES AFTER TREATMENT

It is a well-known fact that after the injection of a trypanocide in experimental trypanosomiasis the parasites disappear. If the treatment is then discontinued, the parasites usually reappear after a variable period. In our large experience with *T. gambiense* and *T. brucei* we have been able to observe that, to a certain extent, a regularity prevails in the interval between disappearance and reappearance. In infection with *T. gambiense* in rats and monkeys, this interval usually extends over fifty to sixty days after injection of Atoxyl or Orsudan and discontinuance of treatment. In infections with *T. brucei* in rats, guinea-pigs and dogs, on the other hand, only sixteen to twenty-five days elapse before reappearance. This is a very interesting observation, for which we are at present unable to account. It suggests that Atoxyl may be retained in the organism for such a period, and that only after its entire removal, the multiplication of the very few surviving parasites sets in anew. This discrepancy between the relapsing time after infection with *T. brucei* and *T. gambiense* may be due to a slower multiplication of the latter parasite in comparison with the former. In our therapeutical experiments with p- and m-amino-phenyl-stibinic acid the relapsing time did not generally show the same regularity.

It is possible, on the other hand, that the course of the relapses is determined by the life-history of the trypanosome. Breinl and his co-workers were able to work out a life-history of *T. gambiense* in the warm-blooded host, but after prolonged research were unable to observe any life cycle of *T. brucei*, and could only demonstrate multiplication by simple fission. This observation makes it doubtful if the regularity of relapsing time can be dependent upon a definite life cycle.

### VALUE OF SUBINOCULATIONS

Particular stress has been attributed to the value of subinoculation of peripheral blood from an animal after treatment, in order to decide whether the animal has been cured from the disease. In our opinion, no conclusion concerning a definite cure can be drawn from subinoculation. If subinoculations are made shortly after injections of a

drug, even with large quantities of blood into susceptible animals, very rarely do parasites appear in the blood of the subinoculated animal. The result of subinoculations after a longer interval varies considerably. Even in the case of large animals infected with *T. brucei*, after treatment and temporary disappearance of the parasites, subinoculations are very frequently negative; sometimes, however, an infection occurs after a very prolonged incubation period.

Less reliable still are subinoculations from cases of Sleeping Sickness patients into rats and guinea-pigs.

The following is an illustration of this statement.

From a patient in a comparatively early stage of trypanosomiasis, at a time when parasites could be seen in very small numbers in the peripheral blood, subinoculations were made into a guinea-pig and two rats. The guinea-pig, although examined daily for six months, never became infected after injection of 2 c.c. of blood. Of the two rats, both showed parasites on very rare occasions, and then only in very scanty number (1 to 3 in each coverslip preparation). One showed parasites on 16 days out of 111 days, the other on 14 days out of 14 months; both died from intercurrent diseases. Although subinoculations upon rats were made from the original rats on days when parasites were observed, none of the subinoculated animals ever became infected.

#### WHEN CAN AN ANIMAL BE CONSIDERED TO BE CURED?

Many statements have been made concerning the efficiency of different drugs with regard to the effect of permanent cures in animals. One is naturally inclined to conclude, from a rapid and apparently complete destructive action of arsenic and antimony upon trypanosomes contained in the blood, that the organism is freed from the parasites. However, careful examination of the blood after treatment reveals the fact that only too often parasites reappear again, even after very prolonged periods. We were able with careful daily examination to observe relapses in rats infected with *T. brucei* and treated with Atoxyl after a negative period of 226 days; and in rats infected with *T. equiperdum* and treated with Orsudan as late as 105 and 126 days after discontinuation of the treatment.



Similar observations have been made by Uhlenhuth and Woithe with regard to a dog infected with *T. equiperdum*, subsequently treated with Atoxyl.

In animals infected with *T. gambiense*, the average relapsing time after discontinuation of treatment, as previously remarked, lies between 50 and 60 days; therefore, observation over a very prolonged period is necessary before considering an animal cured. Occasionally, even in an untreated monkey during an infection with *T. gambiense*, longer intervals may occur, during which parasites, in spite of careful daily examination, cannot be detected, and only intercurrent diseases which lower the vitality of the infected organism cause a reappearance of the parasites. Two of our infected monkeys succumbed to an intercurrent pneumonia and pleurisy. In the blood of the animals, although parasites had not been seen for 41 days, trypanosomes in scanty number reappeared on the day before death.

A third monkey, a very large *Cercopithecus callitrichus*, had seemingly recovered easily from an infection with *T. gambiense*. Its weight increased, the blood count was normal, and we were inclined to assume a natural cure in this animal. Subinoculations made upon rats proved negative. After a negative interval of 74 days the animal suddenly became markedly ill. This was due to an abscess of the gum, which spread in a very short time along the upper jaw and led to the death of the animal at the end of three days. On the day previous to death, trypanosomes were seen in the peripheral blood in very scanty number (1 to 3 coverslip preparations). At death only one parasite could be found in three coverslip preparations. Subinoculations into two guinea-pigs and two rats were made after death with fairly large quantities of blood. One guinea-pig inoculated intraperitoneally succumbed to a purulent peritonitis; the second guinea-pig, inoculated subcutaneously, developed an abscess at the site of inoculation, but became infected after an incubation period of 15 days. Both rats, inoculated subcutaneously, developed a typical infection of *T. gambiense*, and succumbed in due time to the disease.

These observations, together with many others made during our experimental work, point to the assumption that the general condition of experimental animals influences to a large extent the results obtained in therapeutical experiments.

### COMPARATIVE VALUE OF EXPERIMENTS ON DIFFERENT LABORATORY ANIMALS

Contradictory statements have frequently been made with regard to efficiency of different compounds used for the treatment of experimental trypanosomiasis. This is due, to a great extent, to the species of animal used for the experiments and to the virulence of the strain of trypanosomes employed.\* Small laboratory animals, as mice, rats, guinea-pigs and rabbits react in different ways to infection with trypanosomes and also towards drugs. The most conclusive results are obtained by using rats, as these animals are very susceptible to infections with trypanosomes, and their reaction is very constant. The relapsing time is fairly regular and in direct proportion to the trypanocidal action of different compounds.

Guinea-pigs are, as Uhlenhuth, Hübner and Woithe and ourselves have pointed out before, very disappointing, in so far as these animals very often die after a short course of treatment for no apparent reason. Rabbits, too, are only to a certain extent reliable. The infection is of a chronic character, and parasites are usually present in an exceedingly small number. Symptoms, such as inflammation of the eyelids, swelling of the ear and nose, although well marked, are generally easily controlled by the different trypanocidal drugs.

It is unsafe to draw general conclusions from experiments with mice, for the use of drugs in the treatment of Sleeping Sickness and cattle trypanosomiasis, as mice tolerate in proportion to the body weight immense doses of different drugs.†

\* Changes of virulence have been noted on a few occasions in the case of *T. gambiense*. On one occasion *T. gambiense* was recovered from the blood of a monkey at the time of a relapse, after a prolonged negative interval. The number of parasites in the blood of the monkey increased very rapidly within two days and killed the animal. On subinoculation into rats it was noticed that these animals succumbed to the infection usually within 3 to 4 days. After a passage through guinea-pigs the heightened virulence of the strain was lost, and it killed rats again in the normal period, namely 40-120 days.

† Compare the following table from Röhl's publication. (Zeitschrift f. Immunitätsforschung, Bd. I, p. 634.) The maximal dose pro Kg. body weight:—

				ATOXYL.			ARSENO-PHENYL-GLYCINE.
Mouse	...	...	...	0.17 gm.	...	...	0.6 gm.
Rat	...	...	...	0.17 gm.	...	...	0.4 gm.
Guinea-pig	...	...	...	0.08 gm.	...	...	0.12 gm.
Rabbit	...	...	...	0.07 gm.	...	...	0.22 gm.
Dog	...	...	...	0.01 gm.	...	...	0.2 gm.
Horse	...	...	...		..	...	0.075 gm.

# REPORTS OF THE TWENTY-FIRST EXPEDITION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

JAMAICA, 1908-1909

## SECTION I

### MEDICAL AND ECONOMIC ENTOMOLOGY

BY

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## PART I

### TICKS AND OTHER BLOOD-SUCKING ARTHROPODA.

#### TICKS (*IXODOIDEA*)

One of the greatest problems which confronts the pen keepers of Jamaica is the eradication or control of those ticks which by their vast numbers have rendered nearly all the grazing districts of the Island insufferable to man and a veritable plague to his domesticated animals. For forty years or so, these pests seem to have been rapidly increasing, and to-day they swarm in incredible numbers and are a menace to the stock-raising industry of the country. The more serious nature of their presence is, however, the fact that at least one of the species is responsible for the transmission of disease from the sick to the healthy animal; and the losses already occasioned by the death of cattle from the disease transmitted by the so-called Texas-fever tick (*Margaropus annulatus australis*) justify the most energetic attempts towards the control if not the extermination of this pest, as well as those species which also cause serious loss by gross tick infestation.

The first investigation of the tick problem in Jamaica was conducted by the late Professor Williams, whose report is published

in the supplement to the Jamaica Gazette\* for October, 1896. This author claims that the disease as witnessed by him was a chronic form of 'Texan fever . . . conveyed from one animal to another through the intervention of the tick.' His recommendations for the control of the ticks will be considered later. Three different kinds of ticks are mentioned by him as affecting cattle:—(1) 'The large blue cow tick, also called the dog tick, *Ixodes ricinus*, the first known tick on this Island and not supposed to be injurious'; (2) 'the silver shield tick *Ixodes scapulatus*'; and (3) a tick 'similar to or identical with specimens of the Texan cattle tick *Ixodes bovis*.' Subsequent researches have shown that *Ixodes ricinus* does not occur in any of the West Indian Islands or in South America, so that it must have been a case of mistaken identity on the part of Professor Williams, and this species has therefore been eliminated from the list of Jamaican ticks. *Ixodes scapulatus* is synonymous with *Amblyomma cajanense* and *Ixodes bovis* = *Margaropus annulatus* var. *australis*.

During the years 1896-7 an endeavour was made by the Institute of Jamaica to secure a large and representative collection of ticks from all parts of the Island, with the view of obtaining the specific identifications, in the hope that such might throw some light upon the fever from which the cattle were then suffering. Many contributions were received at the Museum from various pen keepers, and the whole collection was subsequently forwarded to Professor G. Neumann, Ecole National Vétérinaire de Toulouse. About three years later a list of the ticks of Professor Neumann's identification appeared in the local press, together with the names of the hosts from which they were collected. The species therein recorded are:—(1) *Rhipicephalus annulatus* var. *microplus* from cattle. (This is evidently synonymous with var. *australis*). (2) *Rhipicephalus sanguineus* (from horses and cattle). (3) *Dermacentor nitens* (from horses and mules). (4) *Amblyomma cajanense* (from the horse). (5) *Amblyomma* (?) *coriaceum* (one engorged female only found on grass); this is a doubtful record and should be eliminated from the list of Jamaican species. (6) *Argas americanus* (= *persicus*), from the domestic fowl. With the exception of the Argas, all the rest were

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\* N. S. Vol. XIX, No. 9, pp. 205-220.



found during the recent expedition, and besides these, three additional species were discovered, viz.:—*Amblyomma maculatum*, *A. dissimile* and *Aponomma* sp. Neumann in recent years has given a doubtful record of *Rhipicephalus bursa americana*, so that the total number of known species recorded from Jamaica is ten.

Some observations on the relative abundance of these ticks will be found under the respective species which are recorded in this memoir. But it may be well to state here that the most abundant kind is the Texas-fever tick (*M. annulatus australis*), and next to this in point of numbers is the silver tick (*Amblyomma cajanense*). The former is essentially a cattle tick, though it infests other animals also; while the latter is a more general feeder and although less abundant, is one of the greatest curses to the Island, owing to the fact that it occurs in all its stages among the grass and pastures, almost everywhere attacking man and beast with impunity. The almost total immunity of the Mysore cattle from the attacks of ticks of all kinds was most marked; this was particularly the case at Shettlewood and other places, where this breed of cattle was used for draft purposes. Crosses between the Mysore and other breeds were also much less subject to the attacks of these pests; while Shorthorns, Devons, Herefords, and Creoles suffered most. Indeed ticks show a decided preference for all cattle which have little or no Indian or Spanish strain in their blood; they have apparently a great dislike to animals with short fine hair; hence, probably, the immunity of the Indian and Spanish races.

It is claimed also that ticks show a decided preference for animals in bad or poor condition; and that a small percentage of animals of all breeds are much more susceptible to ticks than others of the same herd living under precisely the same conditions. Freshly imported European breeds suffer terribly, due, as one correspondent puts it, to the 'want of proper care and attention which their better breeding requires; consequently these animals are seldom in really good health, and so gradually become a special prey of ticks.' Horses with long heavy manes and excessively hairy fetlocks are particularly troubled by ticks, but there are instances recorded 'where there are now and always have been horses which never under any circumstances have ticks' (Clarendon district). Mules are particularly free, though, like horses, they are subject to the tropical horse tick (*Dermacentor*

*nitens*). Goats may be said to be practically immune; but pigs are much subject to them, and more especially are the ears of this animal infested by the 'grass lice' or larvae. Dogs also suffer, chiefly from the bites of *Rhipicephalus sanguineus*, though other kinds infest them.

#### SEASONAL PREVALENCE OF TICKS

During the writer's stay in the Island, from the end of November till the end of January, ticks of nearly all kinds were found breeding in profusion; and the commoner species were seen in all stages, including eggs, larvae ('grass lice'), nymphs and adults. The information supplied by the planters points very clearly to the fact that the season in which ticks are most prevalent is during the dry winter months, and that relatively few ticks are found during the rainy season. There is some indication, however, that the seasons may vary in certain parts of the Island, more especially so in the parish of St. Mary and Portland. It may be interesting, if not important from an economic point of view, therefore, to give a tabular statement of the returns which were made in regard to this question:—

PARISH	SEASON DURING WHICH TICKS ARE SAID TO BE MOST ABUNDANT	
	GRASS LICE OR LARVAE	STAGES NOT STATED
St. Ann ... ..	November-April ...	August-April
St. Andrew ... ..	Dry season ... ..	November-April
Clarendon ... ..	Not stated ... ..	November-April
St. Catherine ... ..	Dry season ... ..	November-May
St. Elizabeth ... ..	Dry season ... ..	December-May
St. James ... ..	Dry season ... ..	January and February
Manchester ... ..	Not stated ... ..	November-April
St. Mary ... ..	Dry season ... ..	March-September or during spring and hot weather
Portland ... ..	Not stated ... ..	April and May or 'early part of year'
St. Thomas ... ..	Not stated ... ..	'Spring months'
Trelawney ... ..	Not stated ... ..	August-April
Westmoreland ... ..	Dry season ... ..	Dry season

#### EFFECT OF RAIN OR WATER ON TICKS AND THEIR EGGS

No experiments were made to determine the effect of submerging the eggs of ticks in water. But Hunter and Hooker,\* in their valuable

\* Bull. No. 72, U.S. Dept. Agr. Bur. of Ent., 1907, p. 22.

Report on the North American Fever-tick, have shown that eggs which were submerged for a period of from ten to twenty-four days nearly all hatched; and that 33 per cent. hatched after being submerged for twenty-five days. These experiments were conducted in order to ascertain what effect the flooding of pastures would have on 'the viability of ticks' eggs on the ground.' These authors also proved that grass lice have a remarkable resistance to water; and that 'flooding under some conditions, as, for instance, during a drought, might hasten incubation.'

That there is a diminution of ticks in Jamaica during the rainy season has already been pointed out; but it is clearly evident that this is not brought about altogether by excessive rains. In the light of our present knowledge of the habits of the Jamaican species, one can only conclude that the dry season is more suitable for the development of the ticks than other times.

#### DISSEMINATION OF CATTLE TICKS BY VARIOUS AGENCIES

The dispersal of ticks has been brought about almost entirely by the hosts to which the various species are peculiar; and the importation of infested cattle into various countries has been the sole means, practically, by which these pests have been disseminated over the larger portions of the tropical and sub-tropical parts of the world. Man alone is entirely responsible for this; and it is only during recent years that any steps have been taken to enforce quarantine or to control in any way the movement of live stock on their introduction into a new country. The question of rendering a tick-infested country free from these animals is an all-important one; and even if this were accomplished on a relatively small scale it remains to be seen how best such an area can be protected from re-invasion, as there are certain agencies by which this could be brought about in a small way other than by the careless introduction of tick-infested cattle. A small proportion may be wind borne, though it is doubtful if such an agency would carry 'grass lice' to any great distance from an infested area. Hosts unsuitable to the development of the Texas fever tick, such as dogs, horses, pigs, goats, or other domesticated animals, or even man, could bring this about in a small degree, so that the greatest possible care must be exercised in dealing with this problem. It has been assumed that the 'Bull Frog' (*Bufo marinus*)

may also be the means of disseminating cattle ticks. This supposition cannot, however, hold good, as the writer found no evidence in support of this theory. Not a single tick was found on this animal with the exception of the species (*Amblyomma dissimile*), which is peculiar to this Batrachian. Moreover, these animals do not wander far afield, so that it is positively certain that they are not and cannot have been responsible, in any degree, for the dissemination of cattle ticks. The mongoose has also been accused of carrying ticks from one district to another, and there may be some truth in this statement; but further proof is necessary, all the more so, seeing that this animal seems to be remarkably free from tick infestation. However, such an animal might easily carry a colony of 'grass lice,' in its coat of long hair, to a very great distance before the parasites relinquished their hold. It seems to the writer, however, that he is rather begging the question in discussing these apparently trivial points in regard to both the Bull-frog and the Mongoose. But these were matters to which his attention was frequently called during his stay in the Island; and the best interpretation, in the light of our present knowledge, has been given.

#### THE PREVAILING CONDITIONS UNDER WHICH PASTURES ARE EITHER MOST FREE OR MOST INFESTED WITH TICKS

One of the questions put to the Pen Keepers was: 'Have you made any observations as to the conditions under which pastures are (a) most free of ticks, (b) most infested with ticks?' There were twenty-six replies, of which a tabulated résumé is here appended:—

##### (a) MOST FREE FROM TICKS

10. Pastures free from weeds and bush
  1. Poor land and poor feeding
  1. Common grass
  1. Scarcity of green grass
  1. Guinea grass not fed by stock
  1. Pastures under constant feeding
  1. Pastures in which cattle are kept from June to December
  1. When stock are absent

##### (b) MOST INFESTED WITH TICKS

5. Guinea grass and dirty pastures
  1. Guinea grass
  1. Rich glades with high guinea grass
  1. An abundance of dry grass
  1. Heavily shaded and foul
  1. Pastures in which cattle are kept from January to May
  1. Periodically fed guinea grass
  1. Bushy and hard fed pastures
  1. Clumps of bamboos with trash beneath them
  1. Accumulations of trash in cool shady places



Judging by these statements it is clearly evident that ticks are most prevalent in dirty pastures with weeds, scrub and trash about them. On the other hand, there is abundant evidence that clean pastures are freer from these pests. We must not lose sight of the fact, however, that 'grass lice,' in particular, will always be less evident on clean ground than on weedy or scrubby ground, as under the former conditions they have less opportunity of clinging to the garments of any person who may pass over infested areas. With tall grass and scrub it is different, because the 'grass lice' climb to the higher stems and leaves, and in this way more readily attach themselves to man and thus render themselves more conspicuous.

Shade and a certain amount of moisture appear, however, to be highly essential to the development of cattle ticks, and as such conditions obtain to a greater extent in dirty pastures and among tall guinea grass, there can be little doubt that more 'grass lice' will hatch, and possibly survive for longer periods, under such conditions than in a clean open pasture exposed to the full blaze of a tropical sun.

#### LONGEVITY OF TICKS WITHOUT ACCESS TO A HOST

That various ticks are capable of fasting for very long periods has been proved repeatedly by many investigators in different parts of the world. In Jamaica, however, nothing has been done in regard to this subject, and it remains to be seen how long the two more abundant species may survive without access to a host. This is a most important matter, as it materially affects the methods of control when dealing with tick-infested land. For the Texas fever tick (*M. annulatus australis*) it is essential only to test the duration of life in the larva. The silver tick (*Amblyomma cajanense*), on the other hand, presents a more difficult problem, as the duration of life without access to a host must be determined in all three of its stages—larva, nymph and adult.

The American authors, Hunter and Hooker,\* proved experimentally that the North American fever tick (*Margaropus annulatus*), the typical form of the variety found in Jamaica, can survive, in its larval stage, without a host for a period varying from 49 to 159 days; and we shall probably find that the Jamaican

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\* *Loc. cit.*, p. 25.

variety is capable of surviving for a similar period. Assuming this to be the case, and we add to these data, the period from the time the female drops from the host to the hatching of the larvae or 'grass lice,' we find that the non-parasitic period may range from 93 to 200 days, approximately, so that these data give us important information on the question of tick eradication, in so far, at least, as the Texas fever tick is concerned. But we have little to guide us in regard to the other Jamaican species (*A. cajanense*), though we have ascertained the facts regarding the incubation period of the eggs and other matters relating to the earlier stages of this pest.

Many interesting details could be given here in relation to the longevity of various kinds of ticks found in other parts of the world, but such facts could have no practical bearing on the subjects under consideration.

One authority claims that 'young ticks or "*grass lice*" suck the juice from the young blades of grass and grow and thrive upon it until a better host comes along'; further that the 'young and tender shoots of the springing that follows burning affords an irresistible temptation to young ticks . . . so much so that they can be seen in millions going from adjoining pastures to a newly burnt one.' It is a commonly accepted theory among peasantry also that ticks can live and multiply without an animal host, becoming blood suckers when occasion serves, and though it may appear, in some cases, as if this were founded on fact, the inference is wrong. There is no evidence that ticks of any kind, can survive or mature on a vegetable diet.

#### LIFE CYCLE OF TICKS

Some details concerning the life histories of the Jamaican ticks are given in this Report (pp. 434-446), which, so far as they go, form a basis for economic procedure in dealing with tick-infested pastures.

All ticks undergo a metamorphosis which consists of the following stages:—1, the egg; 2, the larva or 'grass louse' stage; 3, the nymph; and 4, the sexually mature stage.

*Eggs.* These are laid upon the ground in masses, apparently in sheltered spots. At first they are pale brownish-yellow, but eventually change to translucent brown, resembling the colour

of common commercial glue. As incubation advances, a small whitish spot appears beneath the cuticle, evidently formed by the excreta of the embryo. The incubation period varies in the different species, and temperature has evidently a marked effect upon the development of the embryo. These produce:—

*Larvae*, 'grass lice' or 'seed ticks.' All larvae are six-legged; and the sexes are not distinguishable. On hatching from the egg they crawl up the stems of grasses and other plants, usually to the topmost leaf or stem, congregating together, sometimes in enormous numbers. The 'clusters' or 'nests' are as a rule the progeny of one parent. They remain in such situations for a host; meanwhile no kind of food is taken as a substitute for blood; and they are capable of fasting for very long periods, though it is highly probable that nature provides them with a store of food which may tide them over the first few weeks. When a host is secured they take a meal of blood, filling and distending their bodies considerably. The first moult takes place afterwards, and the eight-legged or nymphal stage is reached.

*Nymph* (Pl. XIII, figs. 1, 2). In this stage also there is no sexual distinction. The animal has now increased in size, though it may be, and often is, smaller than a fully engorged larva. Again the tick fills itself to repletion, the body becoming greatly distended during the process. Shortly after feeding, the nymphal skin is cast and the sexually mature stage is reached.

*Adult Tick* (Pl. XIII, figs. 1, 2). The sexually mature male and female are often identical in size immediately after the change from nymph to adult, and females of small size may frequently be found *in coitu* (*Margaropus annulatus australis*). Little change takes place in the male after feeding; but the female in filling her body to repletion becomes enormously distended, increasing in size from thirty to forty diameters. She falls from the host when fully engorged, and after the lapse of a few days begins to lay her eggs. This process is continued over several days; and in the end the female dies, leaving her body attached to the little mass of eggs. This is briefly the metamorphosis of the cattle ticks, and the cycle is practically the same in all other known species of this division of the Ixodoidea. In habits, however, they vary considerably: some species require but one host, such, for instance, as the cattle tick of Jamaica

(*Margaropus annulatus australis*); others have two hosts; and, like the silver tick (*Amblyomma cajanense*), others again require three hosts. With the first named, all the meals and both moults are effected on the same animal. The second kind moults on the first host as a larva, and leaves the host as a fully engorged nymph, moults for the second time on the ground, and when sexually mature seeks another host. The last named undergoes both moults upon the ground; the first as a larva, the second as a nymph, so that in this way three different hosts are required. With such ticks as these, there are three non-parasitic periods, and for this reason the complete life-cycle must often be greatly prolonged in the absence of a host, and the probabilities are that large numbers perish for the want of food. With the fowl tick (*Argas persicus*) the parasitic periods, with the exception of that of the larva, are all of short duration, and the female may live for a very long period producing many batches of eggs at irregular intervals.

#### STRUCTURAL CHARACTERS OF TICKS

These animals are divided into two families: 1, the Argasidae; 2, the Ixodidae. There are representatives of both groups in Jamaica, though the prevailing forms belong to the second division. It is not intended to enter fully into the structural details, but to point out some of their more salient characteristics.

*Argasidae.* Distinguished chiefly by the absence of a scutum or shield on the upper surface of the body. The fowl tick (*Argas persicus*) is the only representative of this group hitherto found in Jamaica.

*Ixodidae.* All the members of this family are provided with a shield or scutum (fig. 1) and the mouth parts (capitulum, figs. 1, 2) project in front of the body. The males differ from the females in having nearly the whole of the upper parts of the body covered with a shield; in the females this is not so; in some species the shield is extremely small, especially so in the genus *Margaropus*, in which it is scarcely visible in the engorged female. With the exception of *Argas persicus* all the other known species found in Jamaica belong to this group. The structural details of one of the members of this family (*Amblyomma cajanense*) are shown in the accompanying



figures (1 and 2), but these can be seen only by the aid of a microscope. The various phases in the development of the Texas fever tick and the silver tick are illustrated on the accompanying Plate XIII, figs. 1, 2.

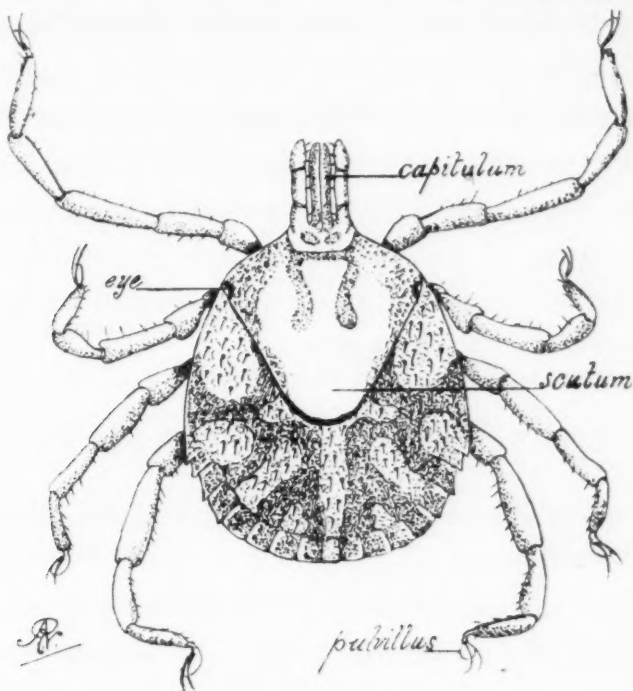


FIG. 1. Silver Tick (*Amblyomma cajanense*). Unfed female. Enlarged ten diameters, about.

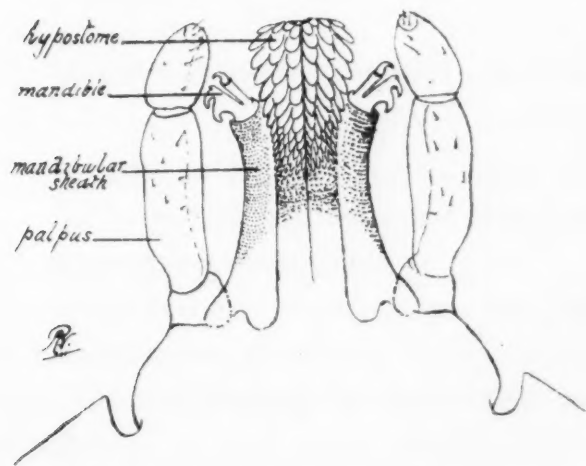


FIG. 2. Mouth parts (capitulum) of Silver Tick (*Amblyomma cajanense*). Ventral aspect. Greatly enlarged.

#### ARTIFICIAL KEY TO THE JAMAICA TICKS

The subjoined key to the ticks found in Jamaica is decidedly artificial in its arrangement. It has been devised as a ready means of assisting in the identification of ticks without recourse to a micro-

scope, though a pocket lens will be needed in order to determine the sculpturing of the integument and those other characters which are embodied in the synopsis. It has not been possible to devise a key for the identification of the various stages in the development of the young adult females, as these vary so greatly in size and shape that it would be impossible to do so with any degree of accuracy or without entering into minute microscopical characters which for the most part are visible only after careful and somewhat tedious preparation. The exact determination of the closely allied species of *Rhipicephalus* cannot, however, be made without a very careful study of the minute anatomical characters by an expert.

#### KEY TO THE NINE SPECIES OF TICKS FOUND IN JAMAICA.

- A. Scutum or shield absent. Sexes similar. Distension after feeding slight in both sexes.
  - 1. Colour chocolate brown; body flat; skin with numerous oval and round discs - - - *Argas persicus* (p. 434).
- B. Scutum present. Sexes dissimilar. Distension after feeding great in female, very slight in male.

#### MALE

*Upper surface yellowish brown, reddish brown or pitchy brown.*

- 2. Mouth parts angulate at sides; ventral surface behind the anus without a median furrow - - -  
*Margaropus annulatus australis* (p. 435).
- 3. Mouth parts similar to 2; ventral surface behind anus with a well-defined median furrow; scutum covered with fine but well separated punctations - - -  
*Rhipicephalus bursa*.
- 4. Mouth parts similar to 2; ventral furrow as in 3, scutum with large and small punctations distributed regularly over the whole surface - - -  
*Rhipicephalus sanguineus* (p. 437).
- 5. Mouth parts *not angulate* at sides; scutum pitchy black, with a median row of punctations on posterior half - - -  
*Dermacentor nitens* (p. 439).

*Upper surface variegated.*

6. Dark rich red-brown markings on a paler ground forming a mask-like pattern - *Amblyomma maculatum* (p. 443).
7. Pale brownish yellow with silvery white markings - -  
*Amblyomma cajanense* (p. 440).
8. Yellowish with yellow-brown spots and radiating lines -  
*Amblyomma dissimile* (p. 445).

## REPLETE OR ENGORGED FEMALE

B. I. *Scutum very small, uniformly red-brown; body greenish or leaden-grey.*

1. Mouth parts angulate; body slightly glossy; pale to dark olive green, sometimes yellowish - -

*Margaropus annulatus* var. *australis* (p. 435).

2. Similar to 1. Scutum covered with fine but distinct punctations - - - *Rhipicephalus bursa*.

3. Mouth parts and colour similar to 1. Scutum distinctly emarginate in front. with large and small punctations. Generally smaller than 1 - - -

*Rhipicephalus sanguineus* (p. 437).B. II. *Scutum uniformly black-brown or pitchy; body uniformly dull yellow or drab yellow.*

4. Integument of body with minute obscure spots - -  
*Dermacentor nitens* (p. 439).

B. III. *Scutum with large coppery spot at apex.*

5. Colour of body similar to 4. Spots on integument generally visible to naked eye - - -

*Amblyomma dissimile* (p. 445).B. IV. *Scutum variegated.*

6. Scutum with silvery grey markings; colour of body resembling 1, but with distinct though somewhat suffused purple-brown reticulations - -

*Amblyomma cajanense* (p. 440).

7. Scutum with dark red net-like pattern; body leaden grey.  
*Amblyomma maculatum* (p. 443).

8. Adult *Aponomma* sp. Not discovered (p. 447.)

All these show conspicuous bright yellow markings after leaving the host and during the period of egg laying.

## CHICKEN-FEVER TICK

*Argas persicus* (Oken).

The larval stages of this Argasid were found 'adhering to the skin of the common fowl' at Kingston, by Dr. J. W. Plaxton, in or about the year 1896. These examples were identified by Neumann, and subsequently recorded in the local press, as then forming part of the local collection in the Museum of the Institute of Jamaica (No. 4,165). A thorough search for this tick was made in several localities without discovering any trace of it—neither were specimens received from any part of the country, though one frequently heard of a 'fowl-tick,' but whether it was of this kind or one of the common cattle ticks of the country is not clear; but seeing that it has already been recorded, the probabilities are that it will be found in many localities, though it may prove to be somewhat local in its distribution.

The bites of this tick are dangerous, and are said to cause prolonged sickness in man in Persia. It is also the cause of Spirochaetosis in fowls, acting as the intermediary host of *Spirochaeta marchouxi*, Nuttall. Like the other members of the sub-family to which it is related, it is not a permanent parasite, though it remains on the host for a few days when in its larval or young stage, in which it is generally known as a 'seed tick.' It is strictly nocturnal in its habits, and after casting its larval skin feeds only at night, leaving the host immediately after taking a meal of blood, seeking shelter in any hole or crevice in the fowl-roost into which it can wedge itself. In these respects therefore it very closely resembles the common bed-bug (*Cimex lectularius*). Unlike the cattle ticks, the female does not die after laying her first batch of eggs, but often survives for long periods, taking frequent meals of blood when the host is available, and laying eggs at irregular intervals throughout life. They can be reared in this country (England) if placed in a suitable temperature; but great numbers die off in the dry atmosphere of the incubator.

Hunter and Hooker\* give a great many interesting details regarding the life-history and habits of this species. Full tables are given by these authors on the oviposition as observed at Dallas in Texas, and the figures given show that the number of eggs laid by

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\* Ibid., pp. 45-46.



the females after the first engorgement varied from 32 to 247; after the second engorgement 55 to 199, and after the third engorgement 32 to 454. The incubation period varied from fourteen to sixteen days. Lounsbury\* has given a very full account of the life-cycle and habits of this tick, which should be read by all who are interested in this subject.

#### TEXAS-FEVER TICK

*Margaropus annulatus*, var. *australis*† (Fuller).

*Male*. Dorsum bright red-brown to castaneous or chestnut brown, with more or less regular, branched, black markings. Legs pale yellowish-brown. Venter, in the space between the legs, brown to pale brown or almost colourless. Ventral shields and caudal process, bright chestnut brown. Length 2.25 mm.

*Unengorged female (i.e. at period of fecundation)*. Dorsum with three well-defined grooves; pale ochreous brown clouded with smoky brown. Scutum bright chestnut brown; eyes darker, tinged with dull crimson. Legs ochreous brown to red-brown. Ventral surface pale leaden grey with irregular pale ochreous blotches or markings. Length 2.50 to 3 mm.

*Engorged female, immediately after removal from host*. Dull olivaceous to leaden grey, sometimes with a faint greenish-yellow tinge; yellowish markings not nearly as pronounced as in females at the period of parturition, being almost obscure in some individuals. Ventral surface, paler, especially so on the anterior half, which is usually of a leaden grey colour. Small area surrounding the anus, whitish. Length 10 to 11 mm.

*Engorged female, four days after removal from host*. Body, slightly glossy; pale to dark olive green, often with distinct yellow markings; scutum small, dark red-brown to chestnut brown; legs very pale brown, faintly yellowish beneath towards the tips.

*Habits*. This tick requires but one host during its developmental cycle; though, as in the case of all other known ticks, the female lays her eggs upon the ground and the larvae or 'grass lice' which they give rise to, crawl up the stems and leaves of various plants, &c., congregating together precisely in the same way that other larval

\* Agric. Journ., Cape of Good Hope, Vol. XXIII. No. 3, pp. 261-273. (Illustrated.)

† Professor G. Neumann has kindly confirmed the Author's identification of this species.

ticks do, and in such elevated positions await some passing host. When once the larva has secured a host, it feeds, and after repletion casts its skin and becomes a nymph. In this latter stage it takes another meal of blood, afterwards casting its skin, and this brings it to the sexually mature stage. So that all its meals and both its moults are effected on one host. Pairing also takes place on the host; and in a very large percentage of cases the male takes up a position close to the nymphal female, considerably in advance of the time when the latter undergoes her last moult and becomes sexually mature. This habit seems to be quite general, though almost any number of adult couples may also occur on the same host. As in the case of the 'silver tick' (*Amblyomma cajanense*), no observations were made as to the parasitic period. But it was found that egg laying takes place in from seven to fourteen days after the replete female is removed from the host, and that the larvae or grass lice begin to hatch on the thirty-fifth day and continue to do so till the forty-second day. So that the length of the non-parasitic stage may be given as fifty-six days during the dry season. The number of eggs laid by the female varies from 1,000 to 2,000 approximately.

It is by far the most abundant tick in the Island; and from 90 to 95 per cent. of the species found on cattle were of this kind.

*Hosts.* The ox is the principal host of this tick, both in Jamaica and elsewhere outside the Island. In three instances, mature examples of both sexes were found on the dog, associated with *Rhipicephalus sanguineus*; several adults and nymphs were also found on the horse, but the females from this host were much smaller than those taken either from cattle or dogs. It is generally believed that the larval or 'grass lice' stage will attack any vertebrate animal that comes in its way. It is in this stage that it is such a great pest to man.

*Distribution.* Occurs everywhere in the grazing district throughout the Island. Specimens were obtained in all the parishes; the only locality from which specimens were not received, was Water Valley, a banana property in the Parish of St. Mary, though it was swarming elsewhere in the district a few miles away. So far as one could ascertain it did not occur at a greater altitude than 2,000 to 3,000 feet, but this requires confirmation.

*Distribution outside the Island of Jamaica.* Australia, South America, and several of the West Indian Islands. Very closely allied forms occur also in South Africa and Japan.

#### LARGE RED TICK

*Rhipicephalus sanguineus*,\* Latr.

*Unengorged female.* Dark red-brown; scutum of the same colour with dark red-black or pitchy markings, especially towards the margins. Length 4 to 5 mm.

*Engorged or replete female.* Resembling very closely small specimens of the Texas-fever tick (*B. annulatus australis*). Length 5.50 to 6 mm.

*Male.* Dark red-brown; scutum of the same colour, with intense black or pitchy markings visible under a pocket lens. Length 2 to 3 mm.

*Habits.* The life-cycle of this species has not apparently been studied, but the probabilities are that both moults are passed off the host, so that in this respect it would resemble the silver tick (*A. cajanense*) in its habits. Structurally the members of this genus closely resemble the Texas fever tick (*Margaropus annulatus*), and it is comparatively recently that they have been separated from *Margaropus*. Miss C. Nuttall, Stony Hill, St. Andrew, who kindly forwarded a number of specimens which were taken from her mastiffs, says that 'these ticks are a source of serious annoyance, as they necessitate the grooming of the dogs twice daily. The small flat brown ones attach themselves between the toes, and are generally found in clusters of three or four. Their bite does not seem to cause the dogs much pain, nor do they trouble us and I have never been bitten by them. The grey specimens (nymphs) get into the hair of the dogs and bite chiefly about the neck. They fall off when fed to repletion, but the result to the dog is a lump about the size of a grain of barley with a blood scab at the top, and quite denuded of hair. You may imagine the misery to the dog when it has chanced into a nest of twenty or thirty of this variety, and the appearance of the animal is that of a badly "surfeited" horse. It is some weeks before the hair grows again. The dogs do not appear to have fever, but are

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\* Determined by Professor G. Neumann, to whom the Author extends sincere thanks.



off condition for some days after being bitten. These ticks crawl about and lay their eggs anywhere, if the dogs are not kept well groomed. We have no cattle, and our pigs and fowls and mules and horses do not seem to get bitten.

'I have tried every kind of wash for the mastiffs that could be used on house dogs, and the water for their rubbing down every morning has a small quantity of kerosene oil in it, but this does not seem deterrent.

'A run of land adjoining ours is simply infested with ticks, grass lice chiefly, and to my almost certain knowledge the cattle belonging to the property have not been allowed into that particular place for over eighteen months—the spot being now a complete ruinate: not even goats wander there. . . . Between us and the aforesaid land is a stone wall, but the ticks are in our pasture, which for years has not carried stock of any kind.'

*Hosts.* This tick does not seem at all particular in its choice of hosts; many and widely separated groups of the Vertebrata being attacked. It is common on the ox and dog, and was found also on the horse (at Vale Royal); but outside Jamaica other animals seem equally suitable, as the appended list will show:—Cat, fox and other canines, hare, dromedary, camel, sheep, goat, birds, and also one or two species of the Reptilia.

*Distribution.* In addition to the records from Stony Hill, this species was also found at Albany, from whence twenty-seven adults (mostly males) and eight nymphs were collected from cattle, and one from the horse; both kinds of animals were also infested with silver ticks (*A. cajanense*). It will in all probability be found in other parts of the Island; but as it bears a striking resemblance to the Texas-fever tick it may be easily overlooked. Neumann has previously recorded it (1897) as occurring on the horse and on cattle at Hopewell, Highgate, S. Mary.

*Distribution outside the Island of Jamaica.* In Europe it has been recorded from France, Austria and Italy. It is widely distributed over the whole of the African continent—Algeria, Tunis, Egypt, Abyssinia, Zanzibar, Cape Colony, Congo Free State, Cameroons, Togoland, &c. It has been found also in Madagascar; Persia, India and China in Asia; Antigua and Colombia in America; and also from Queensland in Australia; so that it occurs in all the four continents.



## TROPICAL HORSE TICK

*Dermacentor nitens*, Neum.

*Unengorged female.* Slightly larger than the male. Dark red-brown or orange brown. Scutum or shield, reddish yellow with irregular blackish markings. Length 3 mm.

*Engorged or replete female.* Uniformly dull yellow or yellowish drab. The integument as seen under a lens uniformly covered with minute black specks, rather widely separated. Ventral surface bluish, paler anteriorly. Scutum or shield, mouth parts and legs dark red-brown. Dorsal half of body, with three more or less well defined and equidistant grooves, two of which have origin at the lateral margins of the scutum; besides these there are sometimes present other short and well-defined grooves, but these are more or less irregular. Length 10 to 12 mm.

*Male.* To the unaided eye appears of a uniform dark red-brown (piceous). Scutum, shining dark red-brown to red-brown, with irregular, and somewhat inconstant, intense, broad, black markings forming a coarse reticulation which almost obscures the paler ground colour. Legs red-brown. Length 2.50 to 3 mm.

*Nymph.* Uniformly pale ochreous with dusky suffused markings. Length 1 mm.

*Distribution.* Found somewhat sparingly at Kew Park, Westmoreland; Friendship and Pepper, St. Elizabeth; Kendal and Quebec Park, St. Mary; Stony Hill and Constant Spring, St. Andrew; Great Valley, Manchester; and Halberstadt, St. Andrew. It has been previously recorded also from several other localities, so that it is fairly widely distributed over the Island, though not an abundant species anywhere.

*Distribution outside the Island of Jamaica.* It is rare apparently in the United States; but is common on horses at San Domingo; and Neumann records it from Guatemala, Venezuela, and Porto Rico and Hayti.

*Habits.* Little or nothing apparently is known of the bionomics of this common and widely distributed species.

*Hosts.* Almost exclusively confined to horses and mules, chiefly the former. It usually occurs in little colonies inside the ears, though

it is found also in other natural cavities of these animals. Colonies of this tick were found altogether on eighteen horses; and two or three isolated examples occurred also on the ox and dog.

### SILVER TICK

*Amblyomma cajanense* (Fab.).

*Engorged or replete female.* Surface of skin not highly polished. Dull bluish grey, with dark purplish brown reticulations. The ground colour appearing as pale round spots on the lower half of the body where the brown colour predominates; excretory system showing through the cuticle as pale dull yellow markings; but there is usually a more or less well-defined patch of yellow, often somewhat rectangular in form just behind the scutum from which the branched markings diverge. Ventral surface pale bluish grey with irregular whitish markings. Mouth parts (*capitulum*) pale brown, usually with two minute dark spots at the posterior lateral angles. Scutum, faintly metallic, with two dark brown submedian curved lines; lateral margin, dark brown behind the eyes, terminating before the apex, the latter pale golden-yellow. Eyes pale, almost colourless. Legs pale brown; segmentation paler, appearing as very faint rings. Length 10 to 12.25 mm.

*Unengorged female.* Similar in size to the male but is easily recognised by the small shield (scutum) on the back. It is also less brilliantly coloured than the male; and the marginal festoons are either entirely absent or scarcely visible to the unaided eye. Length 5 to 6 mm.

*Male.* Pale dull yellow or brownish yellow, with irregular silvery white markings or streaks and brown or red-brown spots, forming a definite but complex pattern which gives the dorsal surface (scutum) a somewhat reticulated appearance, though when seen under a lens the spots and streaks do not coalesce. Length 4 mm.

*Habits.* As already stated this tick requires three hosts during its developmental cycle. The first host in its larval or 'grass louse' stage, the second in its nymphal or second stage, and the third and last host in its adult stages. The replete or fully engorged female (Pl. XIII, fig. 1) lays her eggs on the ground; these produce larvae or 'grass lice,' which congregate together at the tips of grass and leaves, or

the flowering stems or leaves of any other kind of plant or other suitable object, usually something which stands out prominently above the denser vegetation. One enormous colony of 'grass lice' was found clustered together on the flowering stem of a plant locally known as the 'devil's riding whip.' In cattle pens where there is little vegetation they congregate on the exposed roots of trees or swarm up the tree trunks and fix themselves upon any little prominence they may find. Sometimes, also, they occur on stone walls or on angular fragments of rock, or even dead leaves and bits of sticks lying upon the ground. In such situations they await a passing host. When once the young tick has secured a host, it fills itself to repletion, afterwards leaving the host and undergoing the first moult on the ground, becoming a nymph or 'Redback.' In this stage it seeks a second host, usually from the top of some prominent grass stem or other plant, and after taking a meal of blood falls to the ground, moults, and becomes sexually mature. The males and females ascend the taller plants and for the third time seek a host. Both sexes take a meal of blood, and the female after repletion falls from the host, lays her eggs and dies. What eventually becomes of the male is not known. Neither was it ascertained if coition takes place on the host as in other allied species of *Amblyomma*. The females invariably preponderated, and, although enormous numbers of males were seen, in no instances were they found in coitu. The male is extremely active throughout life—mealtime excepted—and so also is the female while seeking a host, but after feeding to repletion, like all her congeners, becomes an almost helpless creature and is capable only of moving her body very slowly.

Little can be said with regard to the period occupied by this tick in the duration of its life cycle. The females are most prolific; the number of eggs laid by the female, according to Williams (*loc. cit.*), is 2,000, but Mr. Wortley makes the figures 7,240, and these may be taken as nearer the average than those of Professor Williams. Egg laying occupies from seven to ten days; and the period of incubation, in an average temperature of 75° F., varied from forty-three to fifty days. The eggs were kept in the shade, and whether the more or less direct rays of the sun would hasten the hatching of the larvae was not ascertained. Beyond this stage nothing can be said at present; neither is it known for how long this tick can survive



in each of the three stages without access to a host. On man the larva fills its body to repletion in from two to four days. At this stage it looks very like a No. 10 gunshot, and is then easily removed. In several instances one or two nymphs were found associated with the colonies of larvae on plant stems; but both nymphs and adults are, relatively speaking, much scarcer than the 'grass lice' or larvae.

*Hosts.* This species is a very general feeder and is not at all particular in its selection of hosts. It occurs most freely on 'horse-kind' and cattle; but is, generally speaking, most abundant on the former. With the horse or mule it confines its attacks chiefly to the head, though it is often found inside the ears, and sometimes also in other natural cavities, as well as on the flanks, withers, mane and tail; while with the ox (cattle) it may be found attached to any part of the body, generally in company with its more abundant relative, the Texas Fever Tick (*M. annulatus australis*). It may be interesting to add, also, that there are several authentic records of its occurrence on the tongues of young calves, though no specimens were secured from such situations. It is common on the pig especially in the larval or 'grass lice' stage, sometimes completely covering the ears of the animal; but it occurs also on this animal in both the nymphal and adult stages. It is apparently not so common on the dog, though specimens in all stages were taken from this animal. To man it is the greatest pest of the Island, attacking him in all its stages from the larval to the sexually mature males and females. It is a most vicious biter, and when it has gained access to the skin inserts its mouth parts (capitulum), the adults producing an irritating wound followed often by intense itching. As showing the extent to which this tick infests man in Jamaica, no less than twenty-seven adults of both sexes, and swarms of larvae, were taken from the writer after walking through a small native settlement which was surrounded by trees and an undergrowth of scrub and long grass. No stock had been turned into this enclosure, neither was it accessible to them; but numbers of pigs were running about the place in a semi-wild condition, and it was evident, therefore, that these animals were acting as the hosts for this tick. On reaching the unclothed portions of the body they bit freely, and so firmly did they attach themselves that several had to be removed with forceps.

From the Portland district comes the note that 'these insects (*sic*)



are a great pest to horses and mules . . . in fact, they attach themselves to every animal that walks in the pasture—birds, fowls and man. To the latter they cause great irritation to the skin, and if the wounds are scratched they are followed by painful sores.' In this instance the writer refers chiefly to the 'red grass lice,' which, so far as one could gather, consisted almost entirely of larvae and nymphs. The same authority says that the young stages of the tick 'are found mostly on the foreheads of horses and mules; also on fowls and pigs.'

As far as could be ascertained, the goat is almost free from this species; and the Batrachians and lizards were not found acting as hosts.

*Distribution.* Generally distributed throughout the Island in all localities where any of the domesticated animals are kept. It occurs in great numbers up to an altitude of 1,500 feet; but no examples were collected from a greater elevation than 3,000 feet.

*Distribution outside the Island of Jamaica.* Mexico, Central and South America are given as the range for this pest.

#### PIMENTO OR NETTED TICK

*Amblyomma maculatum*, Koch.

*Unengorged female.* Uniformly dark brown; legs slightly paler; scutum whitish with one median and two lateral slightly divergent interrupted stripes. In the middle of the space between these and the angles, are two distinct elongated spots, the markings collectively forming a mask-like pattern. Capitulum, dull red-brown; legs of the same colour, with pale articulations. Scutum, pale bronzy pink; anterior portion, pale red-brown merging into a broad median stripe of darker brown terminating considerably within the apex. On either side of this are two divergent and curved lines of the same colour; these extend to the margin, but the apices are considerably interrupted, forming small confluent spots, especially at the extreme margin; the large angular areas enclosed by these lines have each a large sub-central elongated and clearly defined spot; anterior to these spots are several smaller spots; all the dark spots and lines have their margins suffused with pale bronze-green. Abdomen, above, dark brown with obscure blackish markings; margin distinctly festooned; the festoons and the median dorsal area are deeply and coarsely

punctated. Ventral surface ochreous to pale brown; dark brown posteriorly; area of crescentic groove below anus, paler. Stigmata, pale with a broad median dark brown band.

*Engorged female.* Leadен grey with paler irregular markings; integument without spots.

*Male.* Rich dark brown, shining with a very clearly defined reticulated or net-like pattern produced by the paler ground colour. To the unaided eye the animals appear both striped and spotted.

Mouth parts and legs, dark red-brown; articulations of legs not quite so clearly defined as in the female. Scutum, rich dark castaneous, paler towards the margins, especially posteriorly. The bronzy silvery reticulations being clearly defined, but rather thickly studded with black spots and suffused with coppery green reflections; the reticulations of the remaining portions of the dorsum enclosing well-defined areas; each of the festoons with a pale bronzy silvery spot, some of which are nearly equal to the length of the festoon, others small and scarcely visible; these merge into large dark castaneous areas, giving each festoon an ocellated appearance. Ventral surface pale leaden grey; margins, pale red-brown; the intestinal tract showing through the cuticle as pale greyish and blackish blotches.

*Habits.* Little is apparently known of the life-cycle; but Hunter and Hooker,\* who attempted to rear this species on dogs, say that the larvae matured in six to seven days, and then left the host; that the incubation period of the eggs varied from twenty-six to thirty-one days; and that the larvae which hatched from these eggs in the beginning of September were still living on 1st of March following.

*Hosts.* Found upon 'horsekind' and cattle, and, according to Mr. Harry Jackson, is most plentiful 'between the months of June and October. They attack the ears, tails and manes of the animals; and are worse than the ordinary silver tick, as very often they cause sores in the ears and tail.' In addition to the inside of the ear, other natural cavities are also selected; and it bites man almost immediately after gaining access to the skin, inflicting a painful wound, resembling the pain produced by salt when rubbed into a freshly-cut wound, as the writer can testify from experience.

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\* U.S. Dept. Agriculture, Bureau of Entomology, Bull. No. 72, p. 64.

*Distribution.* The only examples of this rather handsome species were captured by Mr. H. Jackson at Waltham, in the Mandeville district of Manchester, on the 18th January, 1909. Several examples of both sexes were forwarded under the name 'Pimento Tick.' No other examples were seen, though carefully searched for in other districts. This is the first recorded occurrence of this species in Jamaica.

*Distribution outside the Island of Jamaica.* North America, especially near the Gulf Coast; Neumann also gives Mexico, Peru, Brazil, Paraguay, Uruguay, Chili and the Argentine Republic.

#### BULL FROG TICK

*Amblyomma dissimile*,\* Koch.

*Engorged or replete female.* Dull ochreous to yellowish grey; excretory system scarcely showing through the integument in pale yellow but irregular lines. Scutum, chocolate-brown with dull coppery markings, forming a distinct spot at the apex. Integument, with numerous well-defined black spots, which in some individuals are surrounded with pale brownish yellow. Length, 17 to 17.50 mm. Greatest width, 11.50 to 12 mm. Height, 6.50 to 17.50 mm.

*Nymphs.* Scutum, deeply but widely punctate with two sub-median grooves; dull ochreous yellow; posterior margin, as far as the eyes, dark brown; eyes, red-brown. Capitulum and legs red-brown. Body, leaden-grey dorsally, with the diverticula showing through the cuticle as two irregular branching lines, slightly diverging from the median line a little above the centre; venter, paler at the margins (almost white), especially in front, and there is a large irregular patch of the same colour surrounding the anal orifice. Ventrally a deep, narrow, and well-defined groove extends from the anal orifice to the margin, and there are also two broad deep divergent grooves on either side; dorsum, with a broad median groove terminating near the middle of the back; and there are two irregular depressions on both sides of this.

*Habits.* The replete or fully engorged female is slightly larger than any other species of tick found in the Island, the length of this tick being equal to one-seventh or one-eighth of the total length of its host (see Pl. XIII, fig. 3). No males were found, though very careful

\* Determined by Professor G. Neumann.



search was made for them. Both the nymphs and the females mature very slowly; and it is evident that all three stages (larva, nymph and adult) are passed upon one host; so that in this respect it differs markedly from its congeneric representative, *Amblyomma cajanense*, which requires three hosts and effects its two moults upon the ground. The life-cycle of *A. dissimile*, therefore, resembles that of the common cattle tick (*Margaropus annulatus australis*). Two apparently freshly attached larvae filled themselves to repletion in about one week; but the nymphal stage occupied from four to seven weeks; and three females were fourteen, seventeen and twenty-three days respectively in maturing. When fully engorged they left the host and in all cases buried themselves among the loose damp grass forming the bed at the bottom of the cage in which the toads were kept. Egg laying, commencing on the seventh day, and was continued for seventeen days. The number of eggs laid by one female was 1,784. These unfortunately did not hatch before the members of the expedition left the Island; but they survived the low temperature of the voyage to England, and, although they were not placed in the incubator until some time after arrival, several larvae hatched out in a temperature of 23° C. during the second week in May; so that it is evident that the eggs of this species are exceedingly resistant to cold. The replete female feigns death when disturbed, but crawls about, when left undisturbed, in a fairly active manner.

*Host.* Apparently confined to the common toad or so-called 'Bull-frog' of the Island (*Bufo marinus*, Gravenh.). This tick often occurred singly; but occasionally four or five examples, in various stages of development, were found upon a single host. It has not hitherto been recorded from Jamaica.

*Distribution.* Sparingly in the Montpellier district in the parishes of St. James and Hanover; Vere District, Clarendon; and at Mona, Bertaville, and Constant Spring in the parish of St. Andrew. It may also occur in all districts where the host is found.

*Distribution outside the Island of Jamaica.* Neumann gives Mexico, Guatemala, Nicaragua, Barbados, Columbia, Venezuela, Brazil, Paraguay and the Philippines; and *Bufo marinus* as the only host.



## LIZARD TICK

*Aponomma sp.*

*Nymph.* Bluish grey with the anterior fourth, both dorsally and ventrally, and also a large patch immediately behind the anus, pale yellowish white; the pale colour also extends along the lateral margins on both sides. Legs, pale brown; capitulum, slightly paler. Scutum, slightly darker than the surrounding integument. Length, 5 mm.

The only tick found upon lizards was discovered by Mr. E. Stewart Panton, who, in submitting the specimen for examination, supplied the following information:—‘I was sitting under a mango tree yesterday, when suddenly a lizard—of the purple-tailed *Anolis* species—a mature male, dropped to the ground from the tree, when I noticed a tick adhering to the throat. On picking up the lizard I found that it was somewhat emaciated and quite weak. I may mention that it is the first time that I have ever seen a tick on a lizard. And situated as it was the lizard could not get at it; otherwise it would no doubt have been eaten by its host before it had even reached its present dimensions.’ [Thornbury, Highgate, S. Mary, 14th December, 1908.]

Quite a large number of lizards were examined during the course of the expedition, but none of them were found to harbour ticks in any stage. The species captured were *Anolis maculata* (many); *Meiva dorsalis* (48); *Gecko* sp. (5).

## ENEMIES OF TICKS

Gosse, in his delightful work on the Birds of Jamaica, was apparently the first authority to call attention to the fact that the two native ‘Blackbirds’ (*Crotophaga ani* and *Quiscalus crassirostris*) feed extensively upon cattle ticks; and although he did not find the remains of ticks in the *post-mortem* examinations which he evidently made, yet his evidence is amply conclusive.

Williams\* considers these birds as the greatest friends to the cattle owners, and that they afforded him much amusement, as there

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\* *Loc. cit.*, p. 215.

seemed to be 'an understanding between them and the cattle whereby they are assisted and encouraged to destroy the ticks.' He appeals also for their preservation. There is no need, however to appeal to the pen keepers or the planters for the protection of these birds, as everywhere the people are unanimously of the opinion that the services which these birds render in destroying ticks are incalculable. The observations which were made in regard to the tick-eating propensities of these two species of birds fully confirm the statements given by former writers and the pen keepers, though it would seem that the 'Tinkling Grackle' (*Q. crassirostris*) feeds much more extensively upon them than the 'Parrot-billed Blackbird' (*C. ani*); this assumption is based both upon observations in the field and upon *post-mortem* examinations, the results of which are given below.

#### SAVANAH BLACKBIRD OR 'TINKLING GRACKLE'

*Quiscalus crassirostris*

(Plate XIV, fig. 2.)

This common species was observed usually in small companies, and seems to be generally gregarious, though odd specimens were noticed in many places. It was seen in considerable numbers in the parishes of Manchester, St. Elizabeth, Hanover, Westmoreland and St. James. In all of these places its tick-feeding habits were observed; and in one instance nearly every individual member of a flock was seen picking off the ticks from cattle which were browsing together under the shade-trees in the pastures. These birds will also follow cattle when driven into the pens, and, if undisturbed, will remove the ticks and eat them with apparent avidity, this habit being particularly noticeable in the mornings and evenings. They are remarkably fearless of man, and if alarmed generally fly into the adjacent trees to return again when unmolested or when hunger presses them to do so.

Judging by the few *post-mortem* examinations which were made, their food consists chiefly of insects and ticks; though they feed to a certain extent upon seeds, and are said also to feed freely upon tangerine and sweet oranges (Montpellier and St. Thomas). The

crop of one example was found to contain the remains of such fruit. The details of the food found in seven examples are given below :—

*Locality.*—Knockalva, Hanover, December 12, 1908. Birds procured immediately after leaving cattle in pen, early in the morning.

NO. 1. CONTENTS: 25 Texas-fever ticks (*Margaropus annulatus australis*); 5 fully, the rest partly engorged females.

3 Silver ticks (*Amblyomma cajanense*); all fully engorged females.

13 Geometrid moth caterpillars.

1 Beetle larva (*Geodephaga*).

Remains of a large centipede.

Hairs from ox.

NO. 2. CONTENTS: 74 Texas-fever ticks (*M. annulatus australis*), 3 of which were fully engorged females; the rest, all adult females, slightly engorged.

12 skins of small geometrid moth caterpillars, all apparently of one species.

Posterior third of a centipede.

NO. 3. CONTENTS: 13 Texas-fever ticks (*M. annulatus australis*), all partly engorged.

10 skins of a small geometrid moth caterpillar.

A mass of the fibrous portion of the fruit of the orange.

*Locality.*—Great Valley, Manchester, December 23rd, 1908. Birds procured under similar conditions to the former, but in the late afternoon.

NO. 4. CONTENTS: 32 Texas-fever ticks (*M. annulatus australis*), all partly engorged females.

3 noctuid moth caterpillars ('cut worms'), all of the same species.

5 geometrid moth caterpillars, all of the same species.

NO. 5. CONTENTS: 3 Silver ticks (*A. cajanense*); 2 fully engorged females, 1 partly so.

- 2 Texas-fever ticks (*M. annulatus australis*); one fully engorged female, the other partly so.
- 13 small geometrid moth caterpillars.
- 1 spider, in fragments.
- 1 chrysalis (pupa) of a noctuid moth.
- 34 seeds of *Portulaca halimoides*, L., and minute fragments of small unrecognisable insects.

- NO. 6. CONTENTS: 7 Texas-fever ticks (*M. annulatus australis*), all partly engorged females.
- 64 large dipterous larvae resembling those of the genus *Tipula*.
  - 3 geometrid moth caterpillars and remains of numerous others.
  - 1 pupa of a large Hymenopterous insect, half an inch in length.

- NO. 7. CONTENTS: Chiefly fragments of moth larvae and other insects, with one small pebble.

The total number of ticks found in the six birds was 159. As these were all females, it will be seen, had they been left to mature, that they would have produced between them over 1,000,000 eggs or a corresponding number of young grass lice; so that the value of the Tinkling as a tick destroyer cannot be over estimated. We gather from these records also that it feeds very largely upon the caterpillars or larvae of moths. This being so, it is only fair to assume that it may feed upon some of the various species which are known to be destructive to various cultivated plants. Whether seeds form a part of its regular diet the writer is at present unable to say; in all probability this may be so; but the seeds may have been taken in lieu of pebbles or sand, as was apparently the case with certain other insectivorous birds\* of the Island.

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\* A female Radiolated Woodpecker (*Centurus radiolatus*, Wagl.) which had regaled itself with cockroaches, contained also five of the large black spherical seeds of the Bitterwood (*Picraena excelsa*, Lindl.), about the size of a buck-shot, which were so hard as to almost resist the blade of a knife. *Platypsaris niger* contained seeds of the West Indian Birch (*Bursera gummiifera*, L.), the Green Tody (*Todus viridis*), seeds of *Panicum glutinosum*, S., and other seeds which were not identified. A rather large seed was also taken from the stomach of the common Petchary (*Tyrannus caudifasciatus*, D'Orby).



## THE SAVANAH OR PARROT-BILLED BLACKBIRD

*Crotophaga ani*, Linn.

Plate XIV, fig. 1

Like the European Cuckoo (*Cuculus canorus*) this bird appears to exercise little or no choice in the selection of its food; nauseous plant-bugs being eaten apparently just as freely as those insects which belong to the edible group. Judging from observations in the field one gathers that this bird also feeds freely upon ticks, though not to such a marked extent, as has already been pointed out, as the Tinkling (*Q. crassirostris*). The writer had many opportunities of watching these birds at close range, as a brood of young birds was reared in close proximity to the bungalow in which the laboratory work in connection with the expedition was conducted. Seven old birds took part in rearing the young, and on several occasions they were seen to take ticks (probably *Amblyomma cajanense*) from the heads of the horses which were grazing hard by. Their method of procedure was to walk close up to the animal while grazing, so as to be able to reach the parasite without flying at the animal's head. On other occasions they were seen inspecting a small herd of cattle which were lying at rest beneath some shade-trees. Three examples only were dissected, and the contents of the stomachs are here appended.

*Locality*.—Stony Hill, St. Andrew, January 4, 1909.

- NO. 1. CONTENTS: Almost filled with portions of the nests (cells, larvae and pupae) of the common paper building wasp (*Polistes crinita*); there were also a few skins of moth larvae; and one spinose skin of the larva of a Nymphalid butterfly.
- 1 beautifully coloured beetle (*Homophoea equinoctialis*, Linn.) of the Chrysomelid group, having a yellow thorax, with deep violet wing-cases bearing eight large white spots.
  - 1 weevil (*Rhyncophorus* Coleoptera).
  - 3 specimens of the pupal stage of the bright orange red 'Cotton stainer' (*Dysdercus* sp.).

- 1 small mollusc (non det.).
- 1 purple berry of the noxious Lantana.
- 3 hard brown seeds (non det.).
- 1 Texas-fever tick (*M. annulatus australis*),  
a partly engorged female.

*Locality*.—Stony Hill and Constant Spring, St. Andrew, January 14, 1909.

NO. 2. CONTENTS: Large fragments of the common 'Green stink-bug' (*Loxa flavicollis*, Drury), in both immature and adult stages, the stomach being well filled with the remains of this insect.

NO. 3. CONTENTS · 2 almost perfect examples of the 'Green stink-bug' (*L. flavicollis*) and many fragments of others, the stomach being about half filled with the remains of this insect.

- 1 beetle resembling a small *Harpalus*.
- 2 small grey weevils and a number of fragments of another Rhyncophorous beetle of a dark brown colour.
- 1 spider.
- 1 Texas-fever tick (*M. annulatus australis*),  
a partly engorged female.

These records give us in a small degree the nature of the dietary of this interesting bird; and it is clearly evident that it is practically an omnivorous feeder. The finding of ticks is of economic value; while the discovery of the green 'stink-bug' (*Loxa flavicollis*) is of great bionomic interest. This bug, whose odour is horribly offensive, does not possess any warning coloration; but being of a uniformly green colour is highly protected and difficult to discover when resting among the leafy branches of a tree or shrub. It is common, but not apparently abundant; though it is not infrequently attracted at night by artificial light. The amount of odoriferous matter contained in the stomachs of the birds found to contain the remains of this bug was so offensive as to render the operation of dissection positively unbearable, and the foetid odour was with difficulty removed from

the hands of the operator. Another find of economic as well as of bionomic interest is that of the injurious 'cotton stainer' (*Dysdercus* sp.): a pest which simply swarms in the cotton plantation in the Parish of St. Andrew, where, if the birds were numerous enough, they might do yeoman service in checking the ravages of this insect.

Another record of interest in reference to the food of this bird was made one day in the month of January, while watching the habits of a small family party bringing food to a fully-fledged young one, which had perched itself in a very convenient place for observation, quite close to where the writer was seated. At first one of the old birds was seen to advance with a huge mouthful of something, appearing most like a bundle of dark coloured feathers, which it was seen to procure from the foot of a tree not far away. This object was offered to the young bird and accepted by it immediately; and while it was making a strenuous effort to swallow the dry-looking morsel, a couple of missiles thrown into the tree made it relinquish its hold of the object, which, when secured, proved to be the somewhat mangled remains of one of the huge black 'Witch Moths' (*Erebus argarista*), measuring originally nearly six inches in the wing expanse.

#### THE DOMESTIC FOWL

It is common knowledge amongst the pen keepers of the Island that the domestic fowl feeds to a marked extent upon cattle ticks. The writer had several opportunities of confirming this, and of noting also that where domestic fowls were not kept that 'grass lice' occurred in countless numbers *inside the cattle pens*, collecting together in enormous masses on any convenient platform that would afford them a means of securing a host. The parents of these had evidently dropped from the cattle when the latter were brought into the pen; and in the absence of fowls had brought forth young, without apparently any check. Mr. Stafford Maxwell, one of the many contributors who furnished us with observations on ticks, evidently attaches great importance to this matter, and says that 'by keeping the pens swept and clean and allowing "Indian Game Fowls" to run in the pens they pick up any ticks which fall from the cattle, and the pens are thereby kept free from these pests.'



## LIZARDS

By the introduction of the mongoose some of our contributors have expressed the opinion that this animal has not only exterminated the quail\* and reduced the numbers of insectivorous birds, but that it has also diminished the number of lizards to such a marked extent that the ticks of the Island have increased proportionally as their enemies have been lessened. This is unfortunately true, so far at least as the birds are concerned; but we have no evidence to show that the innumerable lizards which still exist almost everywhere in the grazing districts play any part in the destruction of ticks. That they are of great value in destroying insects generally there can be no doubt, and these, together with the birds and other predaceous animals, may be considered the only forces with which nature has provided us, to work against the foes of man and his cultivated plants and animals. Some attention was given to the food of the ground lizard (*Meiva dorsalis*); but unfortunately the specimens which were procured for dissection came from the outskirts of Kingston, where there are no ticks, so that it was not possible from post-mortem examinations to glean any facts in support of the theory that these animals feed to some extent upon cattle ticks. But it may be of interest to note, however, that a number of captive ground lizards refused under any condition to eat cattle ticks, though no other food was supplied to them during the many days in which they were kept under observation. But although the results in so far as their tick-feeding propensities are negative, yet one gathers from the few post-mortem examinations which were made that, in a state of nature, they feed exclusively upon those insects which are commonly met with in their haunts, such as earwigs, two-winged flies, including the parent of the noxious 'screw worm' (*Chrysomya macellaria*, see also p. 462), noctuid moths, and numbers of the smaller scarabaeid beetles. These observations on the food of the lizard will not, it is hoped, prejudice the Islanders against the economic value of this animal or any of its allies. Further investigation may yet prove that the inference which has been drawn by the pen keepers and others as to the tick-destroying properties of this and other lizards is a valid one.

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\* *Ortyx virginianus*.



'BULL FROG' (*Bufo marinus*)

The faeces of a number of freshly captured examples of this common batrachian were carefully examined in the hopes of finding traces of cattle ticks in them, but none were discoverable. Seeing that the animal is subject to the attacks of ticks, it may be inferred that it does not eat them, or it would readily remove the parasites from its own body. Such a feat would, however, be an anatomical impossibility on the part of the host; and one may assume that it does not act the part of the Samaritan in removing ticks from its neighbour. Several tick-infested toads were kept together for a considerable period, and it was quite evident that no attempt was made by them to remove the parasites from each other. The remains found in the faeces consisted almost entirely of insects; some comminuted, others quite perfect; and by far the larger proportion were of the common brown Scarabaeid beetles known locally as 'Christmas bugs' (*Ligyrus fossor*, Latr., and *Cyclocephala tetrica*, Voct.), which sometimes swarm in the house and on the dining table at night when the lights are on.

ROTATION OF CROPS AS A METHOD OF ERADICATING  
TICK-INFESTED PASTURES

Various plans have been put forward in the United States of America for the eradication of the cattle tick by adopting a system of rotation of crops suited to the farms in certain parts of the country. Such a system, however, is quite impossible in Jamaica, where the pastures are laid down more or less permanently.

RESULTS OBTAINED BY BURNING PASTURES

This was a question which was also submitted to the pen-keepers. The answers to this were, in a very large percentage of cases, that no beneficial results had been obtained by adopting such drastic measures, and, moreover, they nearly all agreed that pastures so treated became more heavily infested, a few weeks afterwards, than before the grass was fired. But the contributors were certainly not unanimous in regard to this question. Two of them claimed that they had obtained 'very good' and 'very satisfactory results' respectively. Many consider the burning of pastures as a ruinous proceeding, as

weeds take the place of grass ; while others, again, adopt the system more or less regularly, though the results so far as the destruction of ticks are concerned are apparently nil.

One correspondent is opposed to the burning of pastures, because of an extremely interesting discovery which he once made in reference to the silver tick (*A. cajanense*). He says that 'in digging a pond, ticks, in crowds, turned up at a depth of 2 feet to 18 inches.' He argues, therefore that if ticks exist under similar conditions in pastures which may be full of cracks in dry weather that burning must be perfectly futile.

Natives, it would seem, are also opposed to the burning of pastures, because, in their opinion, fire causes ticks to breed more freely ; and so firmly is this idea rooted in their minds that it is very difficult to get them to burn even those ticks which are picked off cattle.

Burning, of course, destroys all those ticks which are upon the grass or herbage ; but it is quite evident that the majority of those which are protected in cracks or crevices in the ground or under logs of wood and under stones escape destruction ; otherwise it is impossible that re-infestation could be brought about so rapidly afterwards. The explanation of the failure of fire in the destruction of ticks in pastures is, in the writer's opinion, undoubtedly due to the fact that the ground had not been cleared of all stock for a sufficiently long period to enable the ticks to lay their eggs and for all the grass lice to hatch and disport themselves over the herbage, thereby exposing themselves to the flames and ensuring complete extermination. To obtain thoroughly satisfactory results in the burning of tick-infested pastures, fire should not be applied until the eighth week after the removal of the stock ; and it is scarcely necessary to add that this should be done in the dry season, and that none but clean cattle or 'horsekind' should be turned into the pasture afterwards. It was suggested by one correspondent that in his opinion 'the huge full-grown ticks which fasten on the "frogs,"\* which are found in great numbers at night in burnt pastures,' may be the cause of the re-infestation of grazing land. The tick in question (*Amblyomma dissimile*) is not of any economic importance to the pen-keeper, as it

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\* *Bufo marinus*. See also the tick *Amblyomma dissimile*.

does not attack his stock ; and, moreover, it does not, so far one could gather, occur in sufficiently large numbers to cause any appreciable annoyance to man or his domesticated animals. Further information regarding this tick is given in other parts of this Report (pp. 445, 446).

#### APPARATUS

The terms 'sprays' and 'washes' are practically synonymous, as the agents used in both cases may be identical in composition. But tick-infested cattle must be treated according to the existing conditions or size of the herd. Small owners need go to no expense in the purchase of apparatus, as washes can be applied with a suitable brush, a piece of cloth, or a bundle of tow ; but for larger herds the pen-keeper will require either a spraying apparatus or a dipping tank. There are numerous forms of spraying machinery on the market, which for the most part are rather costly. For experimental purposes we used a Stot's Syringe fitted with one of their fine nozzles and a Cooper's 'Bucket Spray Pump,' kindly furnished by the inventors. The syringe proved by far the more convenient apparatus ; it was, moreover, extremely economical, there being practically no waste of material. By its use, also, the spraying was carried out expeditiously, and the movements of the animal could be much more easily followed than with a heavier piece of apparatus. The Stot's syringe or hand sprayer costs 10s. 6d. (f.o.b.), the Cooper's spray pump 28s. 6d. (f.o.b.). The best form of brush for liquid washes is the kind used for grooming horses, known technically as a 'body-brush.' A native-made brush, in general use in the Island, is prepared from the fruit stalk of the cocoanut palm ; it forms an excellent apparatus for applying thick tar and oil preparations, but is quite unsuitable for the more mobile washes.

*The use of dipping tanks.* This is undoubtedly the most efficacious means of treating tick-infested stock, as has been abundantly proved in other parts of the world. Unfortunately, dipping is out of the question in some localities owing to the scarcity of water at the time when stock most require dipping. But there are certain parts of the Island where public dipping tanks could be erected, and there are certain estates on which it would repay the owners to erect such a structure for their own use exclusively. This



is a question, however, which the pen-keepers can best settle among themselves, and one also which has been brought forward recently by the Government and the Agricultural Society, through the generous offer of Messrs. Cooper and Nephews to supply the apparatus free of cost to the Island.

#### CATTLE WASHES AND DIPS\*

Williams, in his official report,<sup>†</sup> recommended as the cheapest and most reliable dressing for cattle ticks: 'One pint of tar to three pints of boiled linseed oil, to be applied to all parts of the tick-infested skin,' and added that 'if one dressing be not sufficient a second should be applied in a few days.' This formula, with various complicated modifications, has been in more or less general use for the last thirteen years. That this mixture or a modification of it is effective *as a local application* cannot be denied, but it is much too drastic in its effect upon cattle to be of any real service in the treatment of tick-infested animals.

There are also numerous other forms of washes in use, many of them prepared from materials which the settler may have at hand, but these, for the most part, are altogether too complicated and, in many instances, also too costly to be applied on a large scale.

Several proprietary washes and dips are also used; and in many instances with satisfactory results. In the series of experiments which were conducted by us at the Government Laboratory some of the more popular of these were tested, by spraying a number of tick-infested cattle. The results obtained gave a percentage of dead ticks varying from 5 per cent. to 65 per cent.

After prolonged experiments a most effective spraying wash has been evolved, consisting of a mixture of Cooper's 'Dip powder' and Cousin's 'Paranaph.'<sup>‡</sup> The former is a most effective preparation

\* This portion of the Report is contributed *in part* by the Honourable H. H. Cousins, M.A., F.C.S., Director of Agriculture, Jamaica, and his assistant, Mr. E. J. Wortley, F.C.S.

<sup>†</sup> *Loc. cit.*

<sup>‡</sup> The formula of this preparation is as follows:

1.	Soft Soap (Chiswick Imperial)	55.6 %.
2.	Water .. ..	21.7 %.
3.	Naphthaline .. ..	5.2 %.
4.	Paraffin .. ..	17.5 %.



when used as a dip, but was not found sufficiently mobile to use as a spraying mixture, as it does not readily penetrate to the skin, especially in long-coated animals, and this was particularly noted in a young calf which was used for experimental purposes. Cousin's 'Paranaph' is also a proprietary article, consisting of soap-paraffin and naphthaline, devised originally for washing hops and fruit trees in Kent. The proportions of Cooper's dip used by us is the minimum strength recommended for use in dipping sheep and cattle; but in South Africa, where the 'Bont Tick' (*Amblyomma hebraeum*) is very difficult to kill, the powder is used with safety half as strong again. The 'Silver Tick' of Jamaica (*A. cajanense*), though closely allied to the 'Bont Tick,' does not appear to be so tenacious of life as its African relative. Realising the difficulty of obtaining the exact nature of the result of this compound upon 'grass lice,' which from their minute size are rendered almost invisible among the hair of the host, control experiments were made by placing masses of the young lice in muslin bags. These were completely immersed in the dip and afterwards suspended in the open air in a cool place and allowed to dry. Eighteen hours afterwards the 'lice' were found still living but on the third day every tick was dead; so that we may justly claim that this preparation is equally effective for all stages of cattle ticks. The exact formula of this wash is here given.

Paranaph 1 part, water 6 parts.

Cooper's dip 1 packet to 20 gallons of water.

One and a half quarts per head seems quite enough if applied properly. This preparation has proved not only effective in its immediate results, but a most persistent and adherent tick-destroying medium. The cattle which were sprayed by us, although left to graze in pasturage which was positively alive with 'grass lice' for a period of five weeks, had at the end of that time scarcely a live tick upon them. We are sanguine, therefore, that the tick problem in Jamaica can be controlled cheaply and effectively with appliances readily obtainable and usable by any owner of stock; but the following conditions should be rigorously adhered to in spraying or washing cattle:—

1. The application of this mixture, which contains POISON, must not under any condition be applied at a less interval than fourteen

days; and in our experience every five to eight weeks during the winter months is sufficient to keep the cattle practically free of ticks.

2. All sprayings or washings should take place in the early morning; and the cattle should be allowed to dry in the shade before turning out to graze.

3. If cattle have to be driven for any distance they should be allowed to cool before spraying. Driving both before and after should be quiet.

4. Cattle of all ages and also cows in calf may be sprayed. Cows in milk should have the lower portions of their udders sponged before milking on the first day of the spraying.

5. The spray should be so finely distributed that practically none of the liquid drips off the animals treated. To avoid danger the operation should be conducted on a site devoid of grass.

6. All waste products and washings from the apparatus used should be thrown into the drains, or, safer still, into a hole in the ground and covered with a layer of soil.

7. All these instructions are applicable both for dipping as well as for spraying. *But in-calf animals should not be dipped a month or so before calving.*

We have every confidence in recommending this preparation, which, although containing *arsenic* is perfectly safe. The cattle treated by us did not suffer in the least; moreover, Cooper's dip and other arsenical preparations have been in use for several years in Africa, Australia and South America, and although thousands of cattle have been put to the test in all of these countries no loss has been occasioned when the regulations for its use have been strictly carried out.

In the conditions obtaining in Jamaica we do not consider that 'dipping' is a practicable method; it is too costly, consumes too much wash, and is not without risk to stock. We advocate spraying with a wash, as above, possessed of such wetting power that a very fine spray will serve to wet and destroy all the ticks at one operation.

## CONCLUSIONS

1. That the tick responsible for the transmission of Texas-fever is the so-called Texas-fever tick (*Margaropus annulatus* var. *australis*), though experimental proof is needed to confirm this in the Island of Jamaica.
2. That ticks are most abundant during the dry season.
3. That ticks are dispersed from place to place chiefly by the host to which they are peculiar.
4. That rain or temporary flooding with water does not destroy ticks or their eggs.
5. That a relatively large number of young ticks will hatch and possibly survive for longer periods in dirty pastures than in pastures which are free from weeds and scrub.
6. That ticks cannot survive indefinitely and reproduce their species without access to a host.
7. That all natural enemies of ticks should be encouraged in every possible way, and that fowls should be kept in all cattle pens.
8. That in all cases where it is practicable the burning of pastures should not be carried out until the eighth week after the removal of all stock.
9. That tick-infested animals should be thoroughly sprayed or dipped regularly at intervals of five to eight weeks, or at less intervals if found necessary. Local applications being of little use in the destruction of cattle ticks, though useful in destroying those species which infest the natural cavities of the horse and mule.
10. That the effort to destroy the ticks must be a united one; no half measures will serve; all must participate in the work.
11. That the evidence of those pen-keepers who have constantly waged war against this pest is that ticks, on their respective estates, are not nearly as troublesome as formerly. The writer very willingly bears testimony in support of this statement.
12. That the Island Government remove the duty from all materials used in spraying and dipping cattle.



GLOSSARY OF COLLOQUIAL NAMES OF TICKS IN USE  
IN THE ISLAND

NAME.	STAGE IN DEVELOPMENTAL CYCLE.	SCIENTIFIC NAME.
Blood or cattle tick ...	Replete adult females ...	<i>Margaropus annulatus australis</i> and <i>Amblyomma cajanense</i>
Bull-frog tick ...	All stages attached to host ...	<i>Amblyomma dissimile</i>
Constab or red-back ...	Nymph ...	<i>Amblyomma cajanense</i>
Cow tick ...	Replete adult females ...	<i>M. annulatus australis</i> and <i>Amblyomma cajanense</i>
Dog tick ...	Adult females ...	<i>Rhipicephalus sanguineus</i> and <i>Dermacentor nitens</i>
Flatticus ...	Usually unengorged adult females	<i>M. annulatus australis</i>
Cow tick ...	Replete adult females ...	<i>M. annulatus australis</i>
Grass lice ...	Larval or first stage ...	<i>M. annulatus australis</i> and <i>Amblyomma cajanense</i> ; also all other species in this stage
Jamaica tick ...	Replete adult females ...	<i>M. annulatus australis</i> and <i>Amblyomma cajanense</i>
Long red-brown oval tick ...	Engorged nymphs ...	<i>M. annulatus australis</i>
Pimento tick ...	Adults of both sexes ...	<i>Amblyomma maculatum</i>
Red backs ...	Unfed nymphs ...	<i>Amblyomma cajanense</i>
Red tick, small ...	Unfed nymphs ...	<i>Amblyomma cajanense</i>
Red tick, large ...	Engorged nymphs ...	<i>M. annulatus australis</i>
Red grass lice, large ...	Unfed nymph ...	<i>Amblyomma cajanense</i>
Silver tick ...	Male and female (unengorged)	<i>Amblyomma cajanense</i>
Silver backs ...	Male and female (unengorged)	<i>Amblyomma cajanense</i>
Texas fever tick ...	Usually the partly and fully engorged female	<i>M. annulatus australis</i>

MYIASIS IN MAN PRODUCED BY THE LARVAE OF  
*CHRYSOMYIA (COMPSOMYIA) MACELLARIA*

Dr. J. A. Allwood of Kingston very kindly presented to the School three immature larvae of this horrid pest which he extracted from the ear of a Syrian, December 26th, 1909.

Though immature they are undoubtedly referable to this species, and there can be little doubt that the patient had become infested during his short stay in the Island. At Constant Spring and also in other parts of the parish of St. Andrews this insect is one of the commonest of the 'blow flies,' and the foetid carcase of a bird or mammal, placed in the sun, forms an attractive bait for this very handsome though much dreaded Muscid. They are extremely active in their habits; but though repeatedly disturbed by the sweep of the fly-net they would return again and again to the same tempting bait.



On one occasion over twenty individuals were counted while they were frequenting the carcase of a mongoose. They seemed equally common during December and January. Like the allied European *Lucilia serricata*, which it much resembles in habits, it evidently passes its larval stage usually in putrid carcasses, but, unlike the latter, it also infests man as well as his domesticated animals. Fortunately for Jamaica the John Crows (*Cathartes aura*) remove practically all traces of carrion from man's habitation, and in this way, no doubt, very materially check the increase of this pest.

The fly may be readily recognised by the following characteristics :

**FEMALE.** Face and jowls testaceous, the latter clothed with long golden hairs; cheeks golden yellow. Third segment of *antennae* smoky brown, basal segments paler. *Eyes* red-brown, widely separated. *Thorax* brilliant metallic grey-green with three broad, equidistant, black stripes having bluish reflections; scutellum bronzy-greenish blue. *Abdomen* bronzy green with bluish reflections. *Legs* black. Length, 6 to 9 mm; expanse of wing, 9 to 17 mm.

**MALE.** Similar but smaller, and the eyes almost meet in the median line.

#### HORN FLY

*Lyperosia irritans*, Linn.

This is the small black fly which is known in the United States as the 'Horn-Fly,' so named because of its peculiar habit of clustering in masses about the bases of the horns of cattle. Howard,\* who has given a very interesting account of the habits and life-cycle of the insect, points out that there is no foundation for the belief that it damages the horn by eating into it or causing it to decay. Curiously, this extraordinary habit does not obtain in England, nor, so far as the writer's experience goes, does it in certain portions of the Continent of Europe; and the few examples which the writer saw in Jamaica certainly did not resort to the horns of cattle as a resting place. Howard discovered for the first time that the eggs are laid in freshly dropped dung and that the whole cycle occupies about a fortnight. Although the writer has had abundant opportunities for the study of the life-cycle in England, he

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\* Insect Life, Vol. II, p. 1.

has not so far been able to find the larval stages in cattle dung; though the insect has been reared artificially in such materials. In Jamaica, as in Europe, the flies generally confine their attacks to the back and flanks of the cattle, and they show a marked preference for certain animals, such as roans and blacks, though for some unaccountable reason they have been seen to single out a red-coloured cow and leave the rest of the herd in comparative peace. It is a most vicious biter and will follow cattle into the shade in sheds as well as under trees, causing them great annoyance.

The first indication of its presence in Jamaica was made by Mr. Stephen Maxwell, of Elphinstowe, St. Elizabeth, who, in forwarding examples for identification, stated that 'these flies have been noticed here for the first time this year, and are very troublesome.' This locality is in the mountains of the Santa Cruz range. Subsequently the writer met with this insect in several places, never, however, in great numbers; and it would seem at present as if it were more particularly confined to the parishes of St. Elizabeth and Manchester. Dr. Froggatt\* records the occurrence of this pest at Vera Cruz, and says that when leaving this place for Habana, Cuba, that on coming on board the vessel he found 'the cattle were smothered with the blood-sucking Horn-fly.' It is evident, therefore, that this insect is common in some of the other West Indian islands. *As a remedy*, or rather a preventative, against the attacks of the flies the wash recommended for ticks may also act as a deterrent against this insect. In the United States train oil, or train oil with a little sulphur or carbolic acid added, has been found to keep off the flies for a few days; and a spade full of lime thrown upon cow dung will destroy the larvae which may be living in it. (Howard, *loc. cit.*).

#### THE 'STABLE FLY'

*Stomoxys calcitrans* (Linn.).

This is the commonest blood-sucking fly in the Island, and appears to be very generally distributed; and although it usually occurred in small numbers, in one or two instances it was plentiful enough to cause the animals annoyance by its persistent attacks.

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\* Official Report on Fruit and other Pests in various countries, 1907-8, p. 26.

The writer has already traced out the life-cycle of this insect ; \* but some observations which were made in Jamaica may not be without interest as bearing upon the economy of the species. Larvae were found at Stony Hill, St. Andrew, in the month of January. They were feeding, in small numbers, on fermenting stable refuse, but in such portions of it only as contained an *excess of green fodder*. The fully matured ones had crawled several feet away from the refuse and pupated in the soil an inch or so below the surface. Several had congregated together at the foot of a tree growing hard by ; and many pupae were found in the bifurcations of the main roots close up to the trunk of the tree. Flies were bred from some of the pupae, so that there can be no doubt as to the identity of the species.

#### SMALL YELLOW HORSE FLY

*Chrysops costalis*, Fab. (= *C. amazonicus* Rond.)

Representatives of the Tabanid group of blood-sucking flies were not only very scarce but also extremely local in their distribution. This was markedly so, at any rate, during the months of December and January. This *Chrysops* was seen in small numbers in the Port Henderson swamps ; and all the specimens which were taken by the writer were captured by him as they settled upon a native driver. They did not attack the mule which was being driven through this little fly belt ; but they evidently attack horses, as two examples which were presented to the School by Dr. Turton were captured by him while in the act of sucking blood from his horse. It produces little or no noise when flying ; and it settles so quietly as to be scarcely felt.

#### A NEW BLOOD-SUCKING TABANID

*Atylotus jamaicensis*, n. sp. (Newstead)

Thorax grey ; abdomen pale brown ; wings faintly speckled.

*Head.* Face white, pubescence white, *beard* white. *Palpi* creamy white, with mixed black and white hairs, the latter preponderating, the former forming a faint black tip. *Forehead* dull bronzy

\* Journ. Econ. Biol., Vol. I, 1906, pp. 157-166, pl. XII.

Reprinted Ann. Trop. Med. and Parasit., Vol. I, pp. 75-110, pl. V, 1907.



ochreous, hairs black; tuberculate spot, black, shining, especially on the lower half; with a very fine (almost obscure) black hair-line extending towards the vertex; the latter with a median blackish suffused spot. *Eyes*, in life, coppery brown with bronzy and golden reflections; and a faint narrow bronzy green band below the centre. *Antennae* reddish yellow, annulations on third segment intensely black; hairs on first segment mixed black and white, the former forming a black tuft dorsally; second segment fringed apically with black hairs; third with five to six scattered black hairs on dorsal surface of the swollen basal portion. *Thorax*, dark smoky grey, with two broad but almost obscure darker stripes. *Abdomen* pale smoky brown, gradually darkening apically; hairs mixed black and white; apical margins narrow and paler. *Legs* a little darker than the antennae; anterior femora, blackish dorsally for nearly their whole length; the tibiae, apically blackish all round; middle and hind pairs similar, but the black is less intense; tarsi, blackish apically, terminal segments entirely so.

Closely allied to *Atylotus completus*, but is distinguished by its smaller size, and the absence of any abdominal markings. It belongs to the same group which includes also *A. tritus*, Walk. Two females only of this small Tabanid were captured by the writer while in a boat off Port Royal, Kingston, Jamaica, December 1st, 1908. The first was taken shortly after leaving one of the little Cay Islands, the other when nearing the mainland of Jamaica. Both examples made repeated attacks on the naked feet and legs of the writer's companions, and one was eventually captured while in the act of sucking blood. When disturbed they disappeared mysteriously into the inaccessible portions of the boat, re-appearing after a lengthened absence. Though a careful watch was kept, no other examples were seen either on the Cay which was visited or on the main Island. It is remarkable, therefore, that the species should be met with at sea only.

#### SMALL DIPTEROUS FLIES INFESTING MAN

These flies resemble *Ceratopogon* in habits, i.e., flight and in their persistent habits of attacking man. All the specimens were captured by arranging half a dozen native boys in single file and then sweeping the net over their feet. They congregate chiefly round the toes; but



more especially those which have slight abrasions of the skin or small ulcers, dirt, etc. They are also easily attracted by fresh meat and recently killed birds and mammals.

Of these flies there were at least two if not three species; and the colour pattern as seen in life is herein appended.

*Large species.* Head, ochreous; eyes, bronzy with green reflections; large segments of antenna, ochreous; tip, smoky brown. Thorax, shining black with obscure greenish reflections (i.e., bronzy greenish-black). Abdomen, above, shining black to brownish-black; broad basal segment and venter, pale translucent ochreous. Legs ochreous; claws dark brown.

*Small species (A).* Similar to the above, but with the posterior tibia blackish, with pale basal and apical bands and darker tarsi.

*Small species (B).* Similar in size to (A), but all the tibiae blackish.

#### A HIPPOBOSCID PARASITIC ON BIRDS

##### *Ornithoetona erythrocephala*, Leach.

One example of this interesting though apparently common parasite was secured as it flew from the body of a freshly killed Green Parroquet (*Conurus nanus*), at Worthy Park on January 8th. Its flight was strong, and had it not persisted in its attacks on the writer it is doubtful if it would otherwise have been secured. Another specimen of this fly was also captured by Mr. W. Maxwell at Friendship in the Santa Cruz Valley, St. Elizabeth, from the body of a Peregrine (*Falco peregrinus nigriceps*), also in the month of January. This example was very kindly presented to the School. The colour pattern of this insect in life was noted at the time; here is a description of it:—

*Head*, red-brown; eyes, black; thorax, black. *Legs*, femora and hind coxa, above, dark vivid green; anterior and middle coxae pale translucent ochreous. *Abdomen*, with a faint tinge of vivid green, most strongly pronounced on the anal segment. Length, 8 mm. Expanse of wing, 21 mm.

## A HIPPOBOSCID FLY PARASITIC ON BATS

*Trichobius parasiticus*, Gerv.

In the hope of procuring ticks from the indigenous bats of Jamaica a visit was paid to the enormous cave on the estate of the Honourable J. V. Calder, Worthy Park, in the parish of St. Catherine. This was on January 8th, 1909. The most distant cavern was found swarming with bats, the whole roof of this enormous place being blotted out by a fluttering cloud 5 to 6 feet deep, their wings producing a sound like that of a strong wind passing through a forest of trees. Specimens of two distinct species of bats were secured, and as these proved to be 'rareties' in our national collections at British Museum, examples of both kinds have been presented to the authorities. *Chilonycteris parnelli* evidently preponderated, judging by the number of individuals captured; but the fruit-eating *Monophyllus redmani* also swarmed. The latter had evidently carried in enormous quantities of the large fruit of the 'Santa Maria' (*Calophyllum calaba*, Jacq.), and quantities also of the Ginep (*Malicocca bijuga*, Linn.), as both kinds were found germinating in the farthest recess of the cave, a great distance from the entrance. Here in this dark recess also were seen quite a number of flies flitting about the lamps as we moved from place to place. These were of two kinds, a tiny midge-like species and a larger kind resembling somewhat a house-fly in size and colour. Specimens of both were secured, but, unfortunately, those of the black-looking species were lost while returning from the cave. The smaller fly proved to be *Trichobius parasiticus*, one of the Hippoboscidae group belonging to the family Streblidae. Besides those which were secured while on flight, fourteen others were taken from nine of the captured bats. They were extremely active, some of them taking flight when efforts were made to secure them; and they appeared by the artificial light somewhat like the small 'powder-winged' flies of the genus *Aleurodes*. Both kinds of bats harboured these parasites. In life the colour is of a uniformly pale yellowish brown; the wings being pale ochreous-white with delicate pale brownish veins. Length, 1.50 to 2 mm.

## CHIGGOE FLEA

*Dermatophilus (Sarcopsylla) penetrans*

Said to be common and generally distributed over the whole of the Island; but this requires confirmation. It was certainly a serious pest in certain portions of St. Catherine, Manchester and St. Elizabeth. It is not only parasitic on man, but is commonly met with also on pigs, confining its attacks chiefly to the feet between and immediately above the hoofs of the animal (Pl. XV, fig. 2). Pigs are most subject to the attacks of the Chiggoe when they have free access to dry sheltered places in sheds or beneath the native huts, especially where the floor or ground is covered with a thick layer of dust and dirt. Pigs which have their feet more or less constantly in wet litter or mud do not apparently get infested.

The local remedy is to paint the infested parts with Jeyes' fluid, undiluted. Man sometimes also employs the same means for destroying the parasite. As a measure of prevention, pigs should certainly not be allowed to harbour beneath or near the native huts; but it would be difficult to adopt even so small a precaution owing to the indolence of the natives, who, it would seem, would rather stay at home, when the occasion serves, paint their feet with Jeyes' and make a holiday of the event!

## ANTHRAX LUCIFER

This conspicuous and rather handsome fly with a streak of brownish-orange on its wings was frequently seen associated with butterflies, frequenting wet mud at the margins of wayside pools, in the beginning of December. It generally occurred singly, but was swift of wing, dashing away from its haunts with lightning rapidity, so that it was with difficulty that examples were captured. It may be of some economic importance, as are other members of the Bombiliidae, in that the larvae of certain species are known to devour the eggs of locusts. This is not a blood-sucking insect, and does not, therefore, come strictly within the pale of this Report. Its striking similarity to a Tabanid of the genus *Haematopota*, when on flight, was so marked as to completely deceive the writer on more than one occasion. For this reason alone has it been thought desirable to make this record.





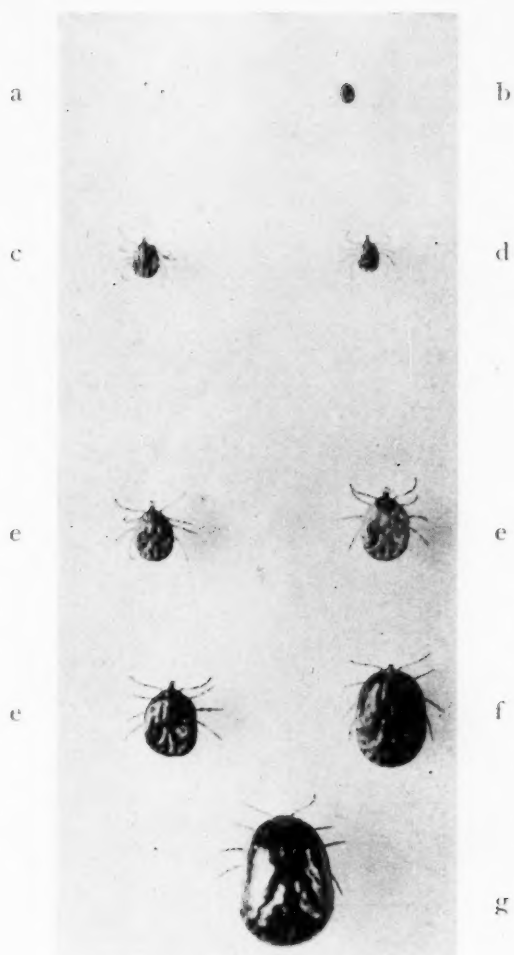


FIG. 1. Silver Tick (*Amblyomma cajanense*).  
Nat. size.

a, Larvae or grass lice; b, Nymph; c, Unengorged female; d, Male; e, Females partly engorged;  
f, Female nearing repletion; g, Replete or fully engorged female.

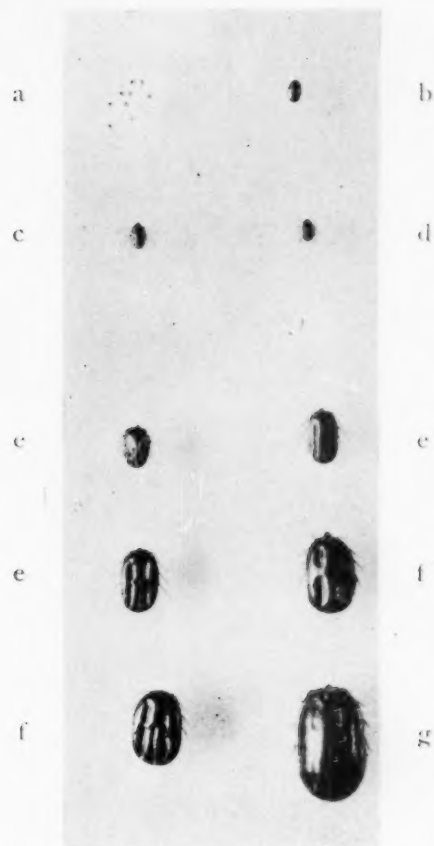


FIG. 2. Texas Tick (*Margaropus annulatus australis*).  
Nat. size.



FIG. 3. 'Bull Frog' (*Bufo marinus*) with the Tick (*Amblyomma dissimile*).  
Nat. size.





FIG. 1. *Crotophaga ani*. Savannah or Parrot-billed Blackbird.



From specimens prepared by the Author.

FIG. 2. *Quiscalus crassirostris*. 'Tinkling Grackle,' or Savannah Blackbird.







FIG. 1. Cattle Ticks taken from the stomachs of two Tinklings (*Quiscalus crassirostris*)



FIG. 2. Feet of the pig infested with Chiggoes (*Dermatophilus penetrans*).



# REPORTS OF THE TWENTY-FIRST EXPEDITION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

JAMAICA, 1908-1909

## SECTION II

## MALARIA

BY

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CONTENTS	PAGE
I. Acknowledgments .....	475
II. Introductory .....	476
III. Description of the Island.....	477
Mountains .....	477
Rivers .....	478
Rainfall .....	478
Temperature .....	478
IV. General History of Malaria.....	478
Various Theories .....	479
Laveran's Discovery of the Plasmodium .....	479
Ross's Discovery of the Mosquito Transmission .....	479
Experimental Proofs .....	480
Rôle of other Mosquitoes in Disease Transmission, Elephantiasis and Filaria, Stegomyia and Yellow Fever .....	480
V. The Nature of Malaria.....	481
The Parasite in the Blood .....	481
Types of Fever .....	481
The Parasite in the Mosquito .....	482
VI. The Anopheles Mosquito.....	483
The Life History of the Anopheles: Eggs .....	483
Larvae .....	483
Pupa .....	483
Adult .....	483

	PAGE
Characteristics of Adult .....	483
Breeding Habits .....	484
Local Conditions under which <i>Anopheles</i> breed in Jamaica .....	484
1. Rivers and swamps .....	484
2. Ditches and gutters .....	485
3. Cattle ponds and pools formed by surface drainage .....	485
4. Rain pools in hollows .....	485
5. Wells occasionally .....	485
6. Drainage trenches in banana plantations ...	485
7. Irrigation canals .....	485
General Mosquito survey required .....	486
Appointment of Government Entomologist advocated .....	486
Principal Varieties of Mosquitoes .....	486
Extract from Old History of Jamaica, Malaria and Mosquitoes .....	487
<b>VII. Malaria in Jamaica.....</b>	<b>489</b>
Clinical Types of Fever .....	489
Varieties of Malarial Parasites .....	489
Prevalence of Malaria .....	490
General Death Rates and Malarial Death Rates .....	490
Sources of Error .....	491
Malarial Death Rates in various parishes .....	492
Admissions to Public Hospitals from Malaria .....	494
Illness among Indentured Coolies .....	495
Return of causes of Illness among Coolies recommended .....	496
Malaria in the Constabulary .....	497
Endemic Index of Malaria—Splenic census .....	498
Absence from school through illness.....	502
Interference with Education .....	502
Summary .....	502
<b>VIII. Anti-malarial Measures adopted elsewhere .....</b>	<b>504</b>
1. Ismailia .....	504
2. Klang and Port Swettenham .....	505
3. Hong Kong .....	506
4. Sierra Leone .....	507
5. Italy .....	507
6. The Panama Canal .....	512
7. Havana (Yellow Fever) .....	513
<b>IX. Anti-malarial Measures in Jamaica .....</b>	<b>515</b>
Measures already in force .....	515
Measures recommended .....	516
Preliminary Observations: Financial .....	516
Agricultural .....	517
Government .....	517
Personal .....	518



Practical Measures—	PAGE
(a) Mosquito Reduction .....	519
1. Rivers and Swamps .....	519
1. Drainage and filling in .....	519
2. Keep outlets to sea open.....	520
3. Clear grass and weeds .....	520
4. Deepen pools .....	520
5. Trenching .....	520
6. Admit salt water .....	520
7. Canalize small streams .....	521
8. Application of Crude Kerosene .....	521
2. Treatment of Shallow ditches and gutters.....	521
Cement drains .....	521
Keep clear of grass and weeds.....	521
Oil .....	521
3. Cattle ponds, etc. Keep clear of weeds.....	521
Deepen the edges .....	522
Oil .....	522
4. Accidental Pools: Fill up or sweep out.....	522
Oil .....	522
5. Wells: Keep clean.....	522
Screen .....	522
6. Drainage Trenches: Keep clean .....	522
Oil .....	522
7. Irrigation Canals .....	522
Legislation .....	522
(b) The Infected Individual (case reduction).	524
1. Mosquito net .....	524
2. Mosquito-proof houses .....	525
Police Barracks .....	525
Public Hospitals .....	525
Coolie Barracks .....	526
3. Quinine Prophylaxis .....	527
(a) Police .....	528
(b) Coolies .....	529
(c) Children: Regular Splenic	
Inspection of Children..	530
(d) General Population .....	531
1. Free Distribution .....	531
2. Ready Distribution .....	532
Quinine Dispensers .....	532
3. Plentiful Distribution .....	532
Quinine during acute attacks.....	533
<b>X. Anti-malarial Organisations.....</b>	<b>534</b>
1. Central Malarial Board .....	534
2. Local Boards .....	534
Staff: (a) Medical Officer's Duties .....	534
(b) Mosquito Gangs' Duties .....	535
(c) Quinine Dispensers' Duties.....	535
<b>XI. Cost .....</b>	<b>535</b>
<b>XII. Necessity for Education .....</b>	<b>535</b>
<b>XIII. Summary .....</b>	<b>537</b>

## TABLES

TABLE I.—Tables showing different zones of elevation in each parish.

TABLE II.—Island monthly rainfall from 1880 to 1905.

TABLE III.—Annual rainfall for each rainfall division in Jamaica.

TABLE IV.—Average annual temperature at different elevations in Jamaica.

TABLE V.—Table showing the death-rate from all causes, the death-rate from malaria, and the proportion of malarial to total deaths.

TABLE VI.—Table showing admissions from malaria in the various hospitals of the Island from 1898 to 1908.

TABLE VII.—Table showing the total admissions from malarial fever in all the public hospitals during 1907-8.

TABLE VIII.—Table showing the number of working days lost through illness on various estates.

TABLE IX.—Table showing the number of cases of malaria among members of the Jamaica Constabulary.

TABLE X.—Table showing splenic rate and average spleen.

### I. ACKNOWLEDGMENTS

I desire in commencing this Report to take the opportunity of expressing my gratitude to His Excellency the Governor, Sir Sidney Ollivier, for his great kindness, for the cordial reception which he gave us, and for the ready way in which he placed the whole official resources of the Island at the disposal of the Expedition. I have also to express my indebtedness to the late Colonial Secretary, the Hon. Clarence Bourne, whose untimely death, alas, occurred while we were in the island. Among other officials who rendered me great assistance, and to whom I desire to express my thanks, I may mention the Hon. Thomas Capper, Superintending Inspector of Schools, without whose assistance the splenic census would have been difficult; Lieut.-Col. Kershaw, the Inspector-General of Police and Prisons, for statistics in connection with the Police and Prisons; the Registrar-General, S. P. Smeeton, I.S.O., who furnished me with valuable statistics as to population, etc.; and the Director of Immigrants, Mr. F. L. Pearce.

I find it difficult to convey a sufficient appreciation of the debt which I owe to the capable and energetic head of the Medical Department, the Hon. Dr. Kerr, who placed every facility at my disposal, and without whose ready aid the work could not have been overtaken. I have also to thank the Medical Staff of the whole Island for their great hospitality and their assistance and help, often rendered, I am afraid, at the cost of very considerable inconvenience to themselves. Among so many helpers, it seems almost invidious to mention names, but I should like to place on record my indebtedness to Dr. Gifford, when Acting S.M.O., Dr. Neish of Spanish Town, Dr. Turton, Dr. Castle of the Public Hospital, Kingston; Dr. Moseley, Dr. Ritchie, Dr. C. H. C. Farquharson, Dr. Edwards, Dr. Hargreaves, Dr. Johnston, Dr. Harvey, Dr. Thompson, Dr. Myers, Dr. McCatty, Dr. Todd and Dr. Malabre.

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I am also indebted to the clergy, who rendered me great assistance, and to the schoolmasters, who gave me every facility

in the examining of children. All others, whom the exigencies of space forbid me to mention, I must ask to accept my most grateful thanks.

## II. INTRODUCTORY

In preparing this report, I have kept two objects in view; first of all, to give as scientific and accurate a description as possible of the prevalence and distribution of Malaria in Jamaica and of the measures necessary to diminish it, which I hope may be of some value and interest to other medical and scientific men who have to deal with similar problems elsewhere; and, secondly, at the same time, to do so in such a manner as will place the facts before the Executive and the general public of Jamaica in simple and non-technical language. For this purpose I have thought it advisable to enter into some details as to the history of malaria, the life history of the parasite, and the habits, etc., of the mosquito, which are now well known to, and accepted by, the scientific world, but which my visit to Jamaica showed me were not fully appreciated by the laity in the Island. Doubtless these details could be obtained from various reports and works, but experience shows that people will not take the trouble to wade through numerous blue books and papers in order to extract the necessary information from a mass of other details, and I hope that in this condensed form it will receive the careful study of those upon whom devolve the various health problems of the Island, and that a serious effort will be made to bring Jamaica abreast of modern progress in this direction. Jamaica has, unfortunately, allowed itself to fall somewhat behind; the sister islands in the West Indies are taking active measures in the direction of anti-malarial and anti-mosquito sanitation, and Jamaica must bestir itself if it is not to be outstripped.

And I should like to say that this condition of affairs is not due to want of appreciation of its necessity on the part of the Head of the Medical Department and the medical profession generally in the Island. One still occasionally meets a medical man who does not believe in the so-called mosquito 'theory' of malaria. If such exist in Jamaica I did not meet any of them. But I did find a body of men, most keen, energetic, enthusiastic, and fully up-to-date in modern scientific methods and in knowledge of the vast strides which



Tropical Medicine has made of late years, and quite prepared to apply these lessons if they received any encouragement. Rather, I think, it must be attributed to a certain amount of indifference on the part of the laity, and perhaps a fatalism due to lack of knowledge that such conditions are remediable; and I am sure that when once the facts are placed before the public, when they once appreciate the importance of dealing with this disease, which interferes so considerably with the prosperity of the island, and with the efficiency, the health and even the lives of its inhabitants, there will be no hesitation in dealing with it promptly and thoroughly.

### III. DESCRIPTION OF THE ISLAND

As there is such an intimate connection between the physical configuration of the Island and the conditions which affect the propagation of the mosquito and thus influence the prevalence of malaria, a brief description of the Island seems advisable.

It is situated between  $17^{\circ} 43'$  and  $18^{\circ} 32'$  North and  $76^{\circ} 11'$  and  $78^{\circ} 20' 50''$  West. The extreme length of the Island is 144 miles and its greatest width 49 miles.

It is very mountainous, a great central chain trending generally east and west, dividing the island roughly into north and south portions. From this chain, a number of subordinate ridges or spurs run to the north and south, there again throwing off other ridges, so that the whole of the central part of the island resembles more than any thing else a huge table-cloth crumpled up into elevations and intervening depressions. Around the coast are flattened areas of varying size, and upon the extent of these and the number of rivers depends to a large degree the prevalence of malaria. (See Table I). The parish of Manchester, for example, with a total area of 302 square miles, has only 42 miles below 1,000 feet, the mountain ridges extending for the most part right down to the coastline. St. Ann, too, with 476 square miles, has only 85 below 1,000 feet. On the other hand, St. Thomas, one of the most malarious parishes in the Island, has 135 square miles out of 274 under 1,000 feet.

The mountains reach a considerable height, the highest being the Blue Mountain Peak, 7,360 feet high, while Sir John's Peak is 6,100, Portland Gap 5,569, Catherine's Peak 5,036, Morce's Gap 4,945, and many are over 3,000 feet.

Jamaica for the most part abounds in rivers and springs, though some parts of the western and midland districts are more or less destitute of water. St. Ann and Trelawny, for example, have no streams of any importance over the greater part of their area, and these parishes are among the least malarious. On the other hand, St. Thomas, Portland and St. Mary are well watered, having numerous large rivers which rush precipitously down from the mountains and spread out into broad shallow streams with swampy margins at their entrance into the sea. During the dry season many of these rivers form comparatively shallow, fordable streams, but owing to the shortness of their courses and the rapidity with which they descend from the hills, they are liable to sudden floods, and in a few minutes a peaceful stream is transformed into a dangerous raging torrent. This is well seen in the Yallah's district, where the river spreads out into a series of shallow mouths communicating with swampy grass-grown pools extending over a large area.

The mean rainfall for the whole Island from 1880 to 1906 was 76.9 inches, but the rainfall varies greatly in different parts, being greatest in the north-eastern division, where the annual rainfall is frequently over 100 inches. The driest months of the year appear to be January, February and March, while the greatest rainfall occurs in May, June and October. (See Tables II and III).\*

The mean temperature varies according to elevation. In Kingston the mean temperature for eighteen years was 78.8, while in the mountains the temperature falls as low as 45°.

As the mountains are inhabited to a considerable height, it is thus possible to obtain a very large variety and range of climate, and in the upper parts it may be described as ideal.

Table IV\* shows the average annual temperature at different elevations.

#### IV. GENERAL HISTORY OF MALARIA

For the benefit of those who are unfamiliar with the history of Malaria, a brief sketch of the principal facts may be of advantage.

Malaria is a disease which has been recognised for many centuries and was known to the ancients as Marsh Fever, owing to its general

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\* Meteorology of Jamaica, by Marshall Hall.

association with swampy ground, but until comparatively recent years nothing was known as to its causation. It has been attributed to many things—to the bad air or miasmata emanating from swamps (hence the name *Mal Aria*), to animalculae, inhaled from the air of swamps, to small vegetable cells, and to bacilli. But the relationship between these various supposed causes and malarial fevers was never proved, and it was not until 1880 that the discovery of the parasite of malarial fever was made. In that year Dr. Laveran, a young officer of the French army working in Algeria, discovered in human blood a minute protoplasmic body, which attacked the red cells, increased in size, developed black pigment, and underwent certain changes, which were found to bear a definite relationship to the stages of an attack of malarial fever.

Confirmation of these observations soon came from different parts of the world. In Italy, India, America, in fact, in every locality in which malaria existed, observers recognised and identified similar bodies, and it was quite clear that, at last, the definite cause of malaria had been determined. But though many additions to the life history of the parasite in the human body were made from time to time, and it was successfully transmitted from one individual to another, by means of injections of blood, for many years the method of its entrance into the human body was unknown. It was evident that it was not infectious, that is to say, it was not communicable directly from one individual to another by contact or proximity. It was thought that possibly the protozoal organisms lived in the water or soil of marshes, that they bred there, developed some form which was capable of aerial transmission and gained admission to the human body by inhalation. Others thought that drinking water was the medium of infection, but no proof was forthcoming of these theories, nor was it found possible to isolate any similar organisms in the soil, water or air of marshes.

In 1895, however, Professor Ronald Ross, then a medical officer of the Indian Army, now of the Liverpool School of Tropical Medicine, began an experimental study of a hypothesis which had been suggested, but which, up to that time, had been purely speculative, namely, that the mosquito was possibly the agent by which malaria was transmitted from individual to individual; and by a series of painstaking and brilliant researches, he succeeded in demonstrating



that the malarial parasite in man was taken up by a special variety of mosquito belonging to the sub-family Anophelinae, and that it underwent a series of definite developments in the body of the mosquito, culminating in the formation of small thread-like bodies, which collected in the salivary glands, and were finally injected along with the salivary juices into the human host, there to give rise to an attack of malarial fever similar to that in the original human host, and characterised by identical parasites.

These results were very soon confirmed by other observers in Italy and elsewhere, and some striking experimental demonstrations of the truth of this 'theory' were given. A number of Anopheline mosquitoes were fed on a patient in Rome infected with a mild variety of malarial fever, and were forwarded to London, where a volunteer, the son of Sir P. Manson, submitted himself to the bites of the infected mosquitoes, with the result that, although living in a locality where endemic malaria is now unknown, and without having lived in any other malarious locality, he developed a typical attack of malarial fever, and parasites, similar to those in the original patient, were identified in the peripheral blood. This experiment has been repeated more than once elsewhere.

Further, Doctors Low and Sambon, living in a mosquito-proof house, in the Roman Campagna, at the most malarious season of the year, remained free from malaria, while all around malaria was rampant among the natives; and that, without any other precaution than that of remaining within the mosquito-proof house from sunset to sunrise, the hours during which the Anopheline mosquito (whose habits are mainly nocturnal) came out in quest of food.

It was evident, then, that an enormous step in advance had been made. It had been found that the principal (and up to the present the only) method by which malaria is transmitted from individual to individual is by means of a particular species of mosquito; and the means of diminishing, and in some instances of actually eradicating, this terrible scourge, which for centuries has devastated the tropical parts of the globe and has rendered so many localities practically uninhabitable, was plainly indicated.

But the attention which had been drawn to the rôle played by mosquitoes in the transmission of malaria, had suggested the possibility of other tropical diseases being carried in the same way,



and it was eventually proved that *Filaria*, a blood worm said to cause Elephantiasis, whose life history in the mosquito had been fully worked out by Sir P. Manson twenty years before, obtained an entrance to the human body through the proboscis of the mosquito, while the brilliant work of American observers in Cuba showed that Yellow Fever was transmitted by means of another species of mosquito, namely, the *Stegomyia*.

The importance therefore of the mosquito as a disease-transmitting agency, and the necessity for its reduction and, if possible, its complete extermination, has been clearly demonstrated.

#### V. THE NATURE OF MALARIA

I have referred incidentally to the parasitic nature of Malaria, but for the benefit of the laity I may outline briefly the life history of the parasite, its mode of propagation and method of transmission, and I shall confine myself to facts which are universally recognised by the medical and scientific world and accepted by them as being definitely proved.

Malaria is caused by the admission into the blood, by means of a mosquito, and so far as we know by that means only, of a minute parasite. If we examine, microscopically, the blood of an individual suffering from an attack, we observe, in certain of the red blood cells, a minute speck of protoplasm, very often associated with small granules of black or dark brown pigment. These parasites gradually increase in size until eventually they involve the whole of the red blood cells. By this time, the parasite has undergone a process of division or segmentation, and consists of a body containing a number of rounded spores. Finally the body bursts and the spores are set free in the liquid of the blood, where they eventually attack a number of fresh red cells, and the process is repeated until the blood gets largely destroyed, and the patient dies or, under proper treatment, the parasites die out.

Three forms of parasites can be distinguished under the microscope, each differing in the duration of the life cycle. The first takes three days to form spores and causes Quartan Fever, the second takes two days to sporulate and produces Tertian Fever, while the third produces crops of parasites at irregular intervals and forms the Malignant types of Fever which are the most dangerous.

Now, as the malarial parasite is unable to leave the human body by the skin, the lungs, the bowels and the kidneys, it is impossible for it to be communicated directly from one individual to another, and it would eventually tend to die out, as has actually happened in many parts of the world.

But Nature, which invariably provides for the propagation of the species under suitable conditions, has here also made provision for the malarial parasite, and, as shown by Professor Ross, a special species of mosquito, the *Anopheles*, steps in as an intermediary.

If we again examine the blood, we find that certain of the malarial parasites do not follow the cycle which I have described and sporulate, but form crescentic or rounded bodies carrying brown pigment, which are easily distinguished under the microscope. These are the Sexual forms. When an *Anopheline* mosquito feeds on an individual having these particular forms in his blood, it draws a number of them into its stomach, where they undergo certain changes, and under favourable circumstances, in about a week, form threadlike bodies or spores which eventually find their way to the salivary glands of the mosquito situated at the base of the proboscis or trunk.

The mosquito has thus become 'infected,' and when it next bites an individual, at the moment when it inserts its proboscis, it injects some of the salivary fluid loaded with spores, which thus obtain admission to the blood, attack the red cells, and the cycle in the human body which I have already described begins once more, and the individual suffers from an attack of Malarial Fever. And thus the vicious circle goes on—man to mosquito, mosquito to man.

It is clear then that for the continuance and spread of Malaria two factors are required, and it is essential to recognise the fact that there are *two*, namely: (1) an individual whose blood contains the sexual forms of malaria, i.e., an *infected individual*; and (2) a species of mosquito, the *Anopheline*, which in its turn must also become infected, i.e. an *infected mosquito*. Do away with one or other or both of these factors and Malaria instantly disappears.

If we have no infected individuals, it does not matter how many *Anophelines* there are in a locality, they cannot become infected, and consequently cannot carry malaria; and conversely, if there are no *Anophelines*, it is immaterial how many infected individuals may be introduced into a locality, the disease cannot be transmitted and must inevitably die out.

And that being the case, it is equally clear, as I shall show directly, that in a locality where Malaria is prevalent, preventive measures against the disease must be directed against either of these factors, or, better still, against both.

## VI. THE ANOPHELINE MOSQUITO

I have mentioned that Malaria can only be transmitted by means of a particular species of mosquito, the Anophelines, and experiments carefully carried out in many parts of the world with the object of growing the malarial parasites in other varieties of mosquito have invariably been unsuccessful, so that we may take it as proved that the Anopheline is the *only* means by which Malaria is transmitted. This fact naturally is of very great assistance in our campaign against Malaria, for the Anopheline has special habits, special characteristics, and selects special conditions for breeding and growth, so that it is advisable that we should be familiar with these.

The life history of an Anopheles Mosquito, as of other insects, consists of four stages:—

1. First of all, the adult female lays its Eggs in water or near it, and in warm weather these hatch out in a day or two to form

2. The Larvae, short, wriggling bodies, which are familiar to everyone as occurring in standing water. They are provided with breathing apertures, and it is important to remember that they *must come to the surface to breathe*. The larvae of the Anopheline Mosquito may be recognised by the fact that, when in repose, they lie *flat* under the surface of the water, while the larvae of Culex and Stegomyia hang head downwards, with their tails and breathing tubes on the surface.

3. After about a week the Pupa is formed, a shorter body with a large head, which also has to come to the surface to breathe.

4. Finally, in two or three days the pupa develops into the adult Anopheline. The latter, when at rest, can generally be distinguished by their characteristic attitude. While the ordinary mosquito stands with its body parallel to the wall, the Anopheline rests with its body in the air, and its head and trunk as if boring down into the wall. It is important to note also that the habits of the Anophelines are mainly *nocturnal*—it comes out as a rule at dusk, and retires at sunrise. It is principally dangerous, therefore, for a limited number of hours.



The Anopheline Mosquito breeds in *shallow still* water or sluggish slowly-flowing streams, and especially where it is *weed* or *grass-grown* or contains green algae at the bottom. This is very important to remember. Thus the larvae will be found in shallow ditches, grass-grown edges of ponds, the shallow margins of streams, badly kept irrigation canals, and so on. It is rarely found in deep water clear of weeds. On the other hand, the Stegomyia or Yellow Fever mosquito breeds in old vessels, broken bottles, barrels, and, in fact, in anything which will hold water.

The **local conditions** in Jamaica under which I have found the larvae of the Anopheline mosquito may be classified as follows:—

1. Along the course and at the mouths of *Rivers*. As already mentioned, these spread out at their entrance into the sea so as to form swamps and shallow pools, all of which are more or less grass and weed-grown. This is well seen at Annotto Bay, where there are no less than three large swamps, formed by the two rivers which enter the sea at that place, and one of these, situated right in the centre of the town, of which a photograph will be found in the appendix, is a typical Anopheline breeding pool. Some of these swamps and pools are almost on the same level as the sea, and are formed by the sea banking up the sand, and thus preventing the outflow of the water. These conditions will be found the most difficult to deal with and improve. It is interesting to note that mangrove swamps do not breed Anophelines, and I once found a curious condition along a road—at one side a mangrove swamp free from Anophelines, and at the other side a grass-grown swamp with numerous larvae. Larvae will breed in slightly brackish water, but very rarely in water that is tidal or contains more than a certain proportion of salt.

In the valleys, along the courses of the rivers, where they spread out, and the stream becomes shallow and sluggish, similar conditions may be found, but wherever the course of the stream is rapid and deep, Anophelines are not to be discovered.

Occasionally, after floods, the depressions in the surrounding flat country become filled up with water and form suitable breeding places for mosquitoes. These are not important unless in the immediate vicinity of towns and villages.



2. The shallow grass-grown *Ditches* along the sides of the country roads, and the earthen gutters at the sides of streets in the towns are also breeding places. Where the surface drains in the towns are cemented, Anopheline larvae are not to be found.

3. Then, scattered about the country, there are a number of larger *Ponds* which are formed by surface drainage, and are chiefly used for watering cattle, and in certain localities, where water is scarce, as the water supply of a village. The margins of these are invariably grass-grown and full of Anopheline larvae. In Great Pedro Bay, where malaria is rife, these are the only sources of the Anophelines.

4. Larvae may also be found in the occasional *Pools* formed in depressions by rain, and I have even found them in the old *hoof-marks* of cattle when left undisturbed for some time.

5. Anopheline larvae are bred occasionally in *Wells*, especially if not very deep, and I have found them, but very rarely, in barrels and tanks used for the storage of water, but the latter are a fertile source of the yellow fever mosquito, the *Stegomyia*.

These may be taken to be the principal *natural haunts* of the Anopheline larvae, but there are other breeding grounds which are artificial and are the direct results of the methods of agriculture in the Island

6. In the banana plantations in the northern part of the Island, where, I am informed, the soil is very heavy, deep drainage is required for successful cultivation. Consequently trenching has to be extensively carried out, and along these *Trenches* a small trickle of water is generally to be found. Now, it was very interesting to me to observe that, wherever I found trenches clean and free from weeds, with a smooth bottom and an even gradient so that no pools could form, no Anopheline larvae were to be detected; but as certainly as I found a grass-grown trench with pools of water, so surely could larvae be discovered. And a well-known planter remarked to me that clean trenches meant good cultivation, so that what is good for the bananas is bad for the Anophelines.

7. In the southern and western parts of the Island, where the rainfall is much less, different conditions obtain. Here both in banana plantations and on some sugar estates, instead of drainage, irrigation

is required, and the smaller *Irrigation Canals*, when not kept clean, were found to be a fruitful source of Anophelines.

I may summarise, then, the principal breeding places in the Island:—

1. Swamps and pools in connection with rivers.
2. Shallow ditches and gutters.
3. Ponds caused by surface drainage.
4. Accidental and temporary pools.
5. Wells occasionally.
6. Drainage trenches.
7. Irrigation canals.

It was impossible for me in the limited time at my disposal to make anything like a complete survey of the various breeding places of Anophelines in different localities, but this has been done to some extent by Dr. Grabham, whose brilliant work on the mosquitoes in Jamaica is so well known, but it is very important that a general survey of the Island should be made, and the various breeding places mapped out. For this and other purposes every Tropical Government should possess an Entomologist.

I also found it impossible to attempt anything like a collection of mosquitoes; but the following remarks, kindly supplied for this paper by Mr. Newstead, Lecturer on Entomology to the School of Tropical Medicine, include a list of the principal species of Anophelines and other disease-bearing mosquitoes:—

' In January, 1905, the total known species of Jamaican mosquitoes ' was twenty-five. Theobald\* gives descriptions of all these together ' with synoptic tables of the sub-families, genera, and species; and ' valuable data, contributed by Dr. Grabham, on the life-history and ' breeding places of these insects. Since the publication of this useful ' memoir seventeen additional species have been added to the list ' by Dr. Grabham, so that the total number of species now recorded ' for Jamaica is forty-two. Little attention was given to the Culicidae ' of the Island by the writer, as it was found altogether unnecessary to ' do so owing to the extensive investigations which Dr. Grabham has ' so ably conducted during the last ten years or so. This authority ' is now in possession of valuable data concerning the bionomics of

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\* The Mosquitoes of Jamaica. Inst., Jamaica, Date Tree Hall, 1906, pp. 1-40.

' the Jamaican mosquitoes, a great deal of which is new and note-  
 ' worthy, and it is to be hoped that he will see his way, shortly, to  
 ' publish the results of his investigations so that students and medical  
 ' authorities may be in possession of facts, which would be  
 ' indispensable in malarial and yellow fever prophylaxis. What is  
 ' most needed at the present moment is a map showing the distribution  
 ' and breeding places of the Anophelines and *Stegomyia calopus*  
 ' (= *fasciata*), especially in those areas in which the towns and  
 ' important villages are situated.

' The Anophelines are represented by four genera and five species,  
 ' of which the following is a list, with the principal localities attached :  
 ' *Anopheles punctipennis*, Say. Port Antonio is the only locality  
 ' given for this mosquito.

' *Cyclolepteron grabhamii*, Theob. Lignanea Plain and Kingston.

' *Arribalzagia maculipes*, Theob. Port Antonio and Morant Bay.

' *Cellia albipes*, Theob. Kingston, the Ferry and Rockfort Swamps,  
 ' Lignanea Plain, Bath, Bowden, Annotto Bay, Port  
 ' Antonio, Bluefields, Castleton and Spaldings.

' This is apparently the most abundant Anopheline  
 ' of the Island, and is said to act as the intermediary host  
 ' of malignant malaria and also of *Filaria bancrofti*.\*

' *Cellia argyrotarsis*, Desv. Kingston only; and, according to  
 ' Theobald, is uncommon, but "acts as the transmitting  
 ' agent of the blood worm *Filaria nocturna*."

' *Stegomyia calopus* (= *fasciata*). Theobald (loc. cit.) says that it is  
 ' "a common insect in Jamaica." It was certainly the  
 ' most prevalent species met with by the writer, and  
 ' seems to be widely distributed in all suitable localities ;  
 ' the greatest number of larvae met with was at Stony  
 ' Hill in the parish of St. Andrew.'

#### MALARIA AND MUSKEETOS 130 YEARS AGO

Before leaving this part of the subject, I am sure that the people  
 of Jamaica will be interested to learn that so far back as 1774 the  
 association of mosquitoes with unhealthiness in Jamaica was  
 recognised, though the actual causal relationship between the two was

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\* Theobald. Ibid. p. 17.



not appreciated. In looking over a very old history of Jamaica, in three volumes, published by T. Lowndes in 1774, a hundred and thirty-five years ago, written by one Edward Long, though his name does not appear on the title page, the following passages occur :—

‘ In the West Indies such low swampy places are still more fatal, and they are infected with muskeetos which seem as if placed there by the hand of Providence to assault with their sting and drive away every human being who may ignorantly venture to fix his abode among them. It is most dangerous to pass the night in such places, and it is at such times that these insects collect in swarms and make war on every daring intruder. . . . Such places in Jamaica are to be deemed unfit for habitation.’

And, later on, the author again remarks :—

‘ It has been observed that muskeetos are intolerably numerous in those places in the West Indies which are least adapted to human habitation. They are found in the greatest swarms among lagoons and swamps on the sea coast, and in little creeks sheltered with mangrove trees; in gullies which contain any stagnant water, in puddles in the flat country after the rainy seasons, and in river courses after the dry weather, where the water rests in detached hollows and becomes corrupted from the fermentation of aquatic weeds and subsided scum. Sometimes I have known them driven from their skulking holes by the violence of strong sea breezes to a considerable distance up the country; but in general among the mountains they are scarce, very diminutive and feeble. They are principally troublesome and in swarms after the periodical rains, when the lowlands are drenched with water and full of little puddles, where these insects deposit their eggs and multiply and breed. . . . These insects cannot exist long nor propagate their species well without stagnant water. Dry weather, dry exposure, and a cool air are equally obnoxious to them; their favourite haunts, therefore, and such as seem most to promote their multiplication, are to be rejected as the least fit (in proportion) for mankind to inhabit, at least during those months of the year when they appear most vigorous and numerous.’

Our old-world author has mentioned nearly every place where Anophelines are now known to breed, and came very near anticipating the mosquito causation of malaria!



## VII. MALARIA IN JAMAICA

*Type of fever.* The clinical types of malaria which exist in Jamaica are the same as are found elsewhere, but so far as my short experience showed me, they are, on the whole, of a milder character than those with which I have come in contact on the West Coast of Africa and in Mauritius. But the severe types are by no means infrequent; there was one death from undoubted Blackwater Fever while I was on the Island, and I obtained the history of several others. Bilious Remittent attacks are also not uncommon.

I examined a number of blood films taken during acute attacks and identified both the parasites of the malignant tertian (aestivo-autumnal of the Italians) and of benign tertian. I was unable to make a systematic examination of a sufficient number of the films to come to any conclusion as to the proportion of the different parasites, but I have to thank Dr. Neish, the Medical Superintendent of the Leper Asylum, who has made special investigations into this subject, for his kindness in placing his figures at my disposal.

He states: 'I have examined over 2,000 blood films. Up to 1905, I have classified these;—the number was 1,636, resulting as follows:—

570 Benign Tertian	72 Double Infections.
384 Aestivo-Autumnal	
79 Mixed	
15 Quartan	
588 Negative	
<hr/>	
1,636	

'All the cases were fever and as far as could be ascertained had not had any quinine. A large proportion of the negative cases had well marked simple tertian symptoms, but there were no parasites in the peripheral blood.

'The benign tertian predominates after the October rains, the aestivo-autumnal during June, July and August.'

It is interesting to observe the considerable proportion of the malignant infections, in view of the fact which I have mentioned,

that the clinical types are undoubtedly milder, and it is an interesting speculation whether parasites having the same morphological characteristics do not exhibit in different countries different strains of virulence. It is also probable that climate and other surroundings affect the malignancy of the attacks, as is seen in cases coming to England from West Africa.

## PREVALENCE OF MALARIA

### *General and Malarial Death-rates.*

Before discussing the question of anti-malarial measures, it will be necessary to endeavour to arrive in some way at the degree of prevalence of malaria, and the extent to which it affects not only the death-rate, but the general health and efficiency of the population, and for the purpose I have prepared a Table (V) showing the total death-rate from all causes for each parish in the Island, the total death-rate from malaria, and the percentage of malarial deaths to deaths from all causes for the ten years ending 30th April, 1907; and I have here to acknowledge my indebtedness to the Registrar-General for his kindness in supplying me with the figures on which this table is based. I had hoped to construct a similar table with reference to the principal towns of the Island so as to arrive at the malarial death-rate of those places, which is very important, because the greater number of the important centres of population are situated on the coastline which is the most malarious, and it would most certainly have been found that the general malarial death rate of a parish is much influenced by the inclusion of those towns. But, unfortunately, the records do not appear to be kept in such a form as to render the figures for the principal towns readily available.

And the configuration of the parishes must also be borne in mind. Without exception each parish reaches to the sea, and has a considerable coastline, the inland part stretching well back into the interior of the Island, and being more or less mountainous. There are no parishes entirely inland. Had it been possible to draw a line round a considerable portion of the interior it would certainly have been found that large tracts of the higher parts of the Island are practically free from malaria, and that the high malarial death-rate

in certain parishes is due to a comparatively limited number of what may be termed plague spots, where malaria is rife; and this information would facilitate very much the application of the anti-malarial measures with which I shall deal presently. But I hope to arrive at this by another method.

Before considering the table, it is necessary for me to point out one or two sources of error. In the first place, no census has been taken since 1891, owing to financial reasons, and, as the Registrar-General rightly points out, the figures relating to population must be taken as approximate and subject to correction at the next census, which I trust will be taken in 1911. I ought also to mention that the slight discrepancy which may be observed between the Registrar-General's figures of the total death-rate and those given by myself are due to the fact that I have calculated the death-rate on the estimated population for the whole year, while he has taken the mean population calculated to the middle of the year.

There is another source of error which must be remembered so far as the malarial death-rate is concerned, and that is that a very large proportion of the deaths, more especially in the outlying parts of a parish are uncertified, and consequently a number of deaths which are registered as being due to 'fever' may be unconnected with malaria. Any illness associated with a rise of temperature, no matter what the cause, is invariably spoken of by the uneducated native as 'fever' and registered accordingly.

So that possibly a more accurate diagnosis might in some cases tend to reduce the malarial death-rate. On the other hand a number of complaints are complicated by malaria, which may be fatal, though the death is returned under the original head, so that this neutralises to some extent the other factor. And, further, the error is a constant one, it will probably occur to the same extent all over the Island and in different years, so that for practical purposes it will afford a very fair standard for comparison.

The table is one of extreme interest, and from it I have calculated the mean death-rates for the decennium 1898 to 1907, which for facility of reference I give below, but I would recommend the serious study of the large table to those who are interested in the health and sanitary condition of their respective parishes.

## Average death-rates, etc., for decennium ending 30 April, 1907

Parish	Average death-rate from Malaria	Average death-rate from <i>Other</i> causes	Average death-rate from <i>All</i> causes	Average percentage of Malarial deaths to Total deaths
St. Thomas .....	6.5	18.5	25.0	26.1
St. Catherine .....	6.2	19.4	25.6	24.4
Westmoreland .....	5.9	15.8	21.7	27.7
St. Mary .....	5.9	18.0	23.9	24.6
Clarendon .....	5.3	15.2	20.5	23.8
Portland .....	5.3	19.3	24.6	21.9
St. James .....	4.8	17.1	21.9	22.3
Hanover .....	4.8	19.5	24.3	19.9
St. Andrew.....	4.0	23.9	27.9	14.6
St. Ann .....	3.4	14.5	17.9	19.4
Trelawny .....	3.4	20.8	24.2	14.4
St. Elizabeth .....	2.9	15.6	18.5	15.8
Kingston .....	2.4	26.3	28.7	8.6
Manchester.....	1.6	14.8	16.4	10.0
Whole Island .....	4.4	18.1	22.5	19.7

It will be seen that for the whole Island the death-rate per 1,000 living, for the ten years in question was 22.5, by no means a high one, as compared with other tropical places. The death-rate attributed to malaria is 4.4 per 1,000, so that had there been no malaria the death-rate for the Island would have been only 18.1. The proportion of deaths attributed to malaria, to deaths from all causes, is 19.7 per cent., so that very nearly *one-fifth* of the deaths in the Island are caused by Malaria.

The mean death-rate from malaria, 4.4 per 1,000, is thus by no means a high one when compared with other malarious localities, for example Mauritius, where Professor Ross found that the average annual death-rate from malaria was 14.0 per 1,000. But the satisfaction which might be derived from this statement is very much modified by the fact to which I have already drawn attention, namely, that Jamaica is largely a mountainous island, that in the higher parts malaria is practically non-existent, and consequently if statistics were available we would find a very low death-rate from malaria over the whole of the centre of the island, and a high one in certain localities along the littoral.



That this is the case is brought out in the table. Manchester, which is an extremely mountainous parish and where the principal centres of population are for the most part situated at high levels, shows the lowest malarial death-rate of all, namely, 1·6 per 1,000.

On the other hand, St. Thomas heads the list with a malarial death-rate of 6·5 per 1,000. Here the local conditions are quite different. A number of large rivers, the Yallahs, the Negro, the Morant, and the Garden, rush sharply down from the Blue Mountains to spread out in the plain below into broad shallow streams with, in many cases, swampy outlets.

And the other parishes which show a considerable malarial death-rate, St. Catherine, Westmoreland, St. Mary, Clarendon and Portland, show much the same conditions, a mountainous hinterland with well-watered alluvial plains devoted largely to banana and sugar cultivation.

It is worthy of note that Kingston, which unfortunately possesses the highest death-rate in the Island, pointing to general insanitary conditions apart from malaria, shows a low malarial rate, the proportion of deaths from malaria being only 8 per cent., and a systematic malarial survey of the town would certainly show that malaria is limited to well defined areas. This immunity is undoubtedly due to the lower rainfall and to the fact that the surface drains are largely cemented, and it would be a comparatively easy matter to banish malaria entirely from the capital of the Island.

The deaths attributed to malaria in the whole island in 1907 were 4,094, while the total deaths from the same cause for the ten years was 34,695, an appreciable factor in the industrial and economic development of the Island.

But the actual death-rate from malaria does not represent fully the amount of that disease; the death-rate will depend upon the particular type of malaria prevalent, and also upon the degree of immunity which is undoubtedly acquired by a native population. They may suffer to a large extent from malaria though they do not die of it, and the extent to which malaria prevails not only affects the general health and physique of a community but interferes with its efficiency for industrial purposes and throws a very considerable financial strain on the colony of Jamaica.

*Hospital Statistics of Malaria*

It is necessary, therefore, to endeavour to obtain some idea as to its prevalence apart from actual death, and this can be done by a study of the hospital returns. Dr. Kerr, the Superintending Medical Officer, kindly obtained for me a series of returns from the various district hospitals showing the number of admissions from malaria from the year 1898 to 1907. These are, unfortunately, incomplete, one or two districts not having furnished full returns, but from the material at my disposal I have constructed Table VI, which shows the admissions to various hospitals for a number of years. Although the grand totals would be considerable, if all the hospitals were included, yet the table brings out one very important fact, that the admissions for malaria have increased very considerably during the past few years, chiefly since 1904-5. I also include a Table (VII) drawn up by Dr. Kerr showing the total admissions from malaria month by month in all the public hospitals of the Colony during 1907-8.

The increase of late years is brought out perhaps in a more graphic way in the following statistics which I have extracted from the Annual Reports of the Medical Department:—

Year	Total admissions from all causes	Total Deaths	Death-rate per cent.	Malarial admissions	Malarial deaths	Malarial death-rate per cent.	Percentage of malarial to total admissions
1904-05	16,103	669	3.7	4,827	89	1.8	29.9
1905-06	17,856	563	3.1	6,285	88	1.4	35.1
1906-07	21,555	661	3.06	7,113	99	1.3	32.9
1907-08	21,837	830	3.8	7,510	121	1.6	34.3
Total	77,351	2,723	—	25,735	397	—	—
Average	19,337	680	3.5	6,433	99	1.5	33.2

It will be seen that the total admissions to the various hospitals from all causes have risen from 16,103 in 1904-5 to 21,837, an increase of over 26 per cent., while the admissions due to malaria rose from 4,827 to 7,510, an increase of over 55 per cent. The total number of cases of malaria treated in the hospitals during the years 1904 to 1908

was 25,735, while the average admissions were 6,433 or 33·2 per cent. of the average total admissions. That is to say, that of all cases admitted to the hospitals of the Colony one-third are due to malaria, and, as Dr. Ritchie of Annotto Bay pointed out in connection with his returns, this does not represent the total amount of malaria, as a considerable proportion admitted for other causes are complicated with malaria.

It is true that the average death-rate from malaria in the hospitals, that is to say, the case mortality, is low (1·5 per cent.), but the importance of this large admission rate lies not only in the loss of labour, but in the great expense to the Colony. Taking the figures from the Annual Report for 1907-8, I find that the total cost of the hospitals (including Kingston) was £19,185 18s. 7d. But a third of the patients were admitted for malaria, so that a third of the cost may be charged against them. In other words, the cost of malaria in 1907-8 was approximately £6,395, and this amount is likely to increase.

From this point of view alone the necessity for energetic anti-malarial measures seems amply indicated.

The increase appears to be largely due, so far as I can judge, to a rise in the number of coolie admissions, and the hospitals which show the largest numbers of *malarial* admissions are those situated in the agricultural districts employing a large number of coolies and which also have the largest number of *coolie* admissions. In more than one Annual Report expressions like the following occur:— ‘Annotto Bay Hospital comes first with 1,780, while Port Antonio runs a good second with 1,611 cases, Lionel Town coming third with 933 cases.’ Statistics as to the number of coolie admissions to hospital will be found in the Annual Report of the S.M.O. for 1907-8.

And as most of the coolies are indentured and are under strict regulations this is a factor which, as I shall show when dealing with the question of prevention, it should be possible to control to a very considerable extent.

#### *Malaria on Estates.*

In order to obtain an idea as to the annual loss to estate owners from sickness among coolies, I requested Mr. Pearce, the Director of Immigrants, to be kind enough to supply me with a number of



statistics, and I have to express my indebtedness to him for the very considerable amount of trouble which he took in the matter. The labour involved made it out of the question to attempt to classify the whole of the estates in the Island, but a number of estates in different districts were selected by me entirely at random, without any personal knowledge of them, some being banana plantations and others sugar estates, and I presume they represent a fair average.

The return included females, but I have only taken into account the male indentured coolies.

Although a very accurate record is kept of the total number of days spent in hospital, no record is kept on the estates of the *nature* of the illness, although this might be obtained, with a great deal of labour, by extracting the information from the hospital records. I would venture to suggest that each estate should keep a record of the nature of the illness for which a coolie is sent to hospital, and this could be done by a simple return to be furnished to the estate by the District Medical Officer. It would be of extreme value in arriving at a knowledge of the prevalence of any disease on a particular estate, and for the purpose of indicating the remedial measures necessary.

But though the number of days lost through malaria are not specifically shown, I think I shall be under-estimating it if I put it at 50 per cent. of the total illness among coolies; in some places it is certainly much more.

I have in Table VIII condensed the information supplied by the Director of Immigrants, and it will be seen that the percentage of days lost through sickness varied from 2·4 to as much as 41·7, the average for 1907 being 15·5 and for 1908 18·3, the average for the two years being 16·9 days lost from illness out of every 100 working days. In other words, 16·9 men out of every 100 on these twelve estates were incapacitated from work daily throughout the year, that is to say, that if it were possible to attain the ideal of no sickness, these estates could have been worked with 16·9 less of a staff. On one or two estates, during certain months *each* individual coolie on the estate spent ten to fourteen days in hospital out of every month of twenty-eight or thirty working days. This, it must be admitted, represents an enormous loss of labour, in addition to what I have already alluded to—the cost to the Colony of maintenance in hospital—and I do not think it will be disputed by any practical business man that if it is



possible to reduce this amount of sickness appreciably, by measures directed against malaria, and at a reasonable cost, it is well worth while giving those measures a thoroughly systematic and determined trial. It will mean eventually that estates can be worked with a smaller staff as satisfactorily and efficiently as they are at present worked by a staff, a considerable proportion of which is permanently incapacitated by illness.

*Malaria among the Constabulary.*

In the Jamaica Constabulary we have a body of picked men who have to attain a certain physical standard before enlistment, who are constitutionally sound at the time of admission to the force, and who live, on the whole, under favourable circumstances as regards food, clothing and housing. They ought, therefore to be a very good index of the prevalence of malaria in any given locality—if they suffer, much more will the general native population suffer.

Colonel Kershaw, the Inspector-General, has been kind enough to supply me with the number of cases of malaria at each station. To get an absolutely accurate idea of the prevalence of malaria in the force, one would have to obtain the number of days off duty from malaria and the proportion of average daily sick from malaria to average daily strength, and no doubt this can be readily obtained if required, but the present return (Table IX) is sufficiently accurate for comparative purposes, and I have only calculated the percentages at the principal stations where there are a considerable number of men, as with small numbers the figures of illness are more liable to error.

Here again we find Annotto Bay occupying the unenviable position of easily heading the list. Every man stationed at Annotto Bay has on average six to seven attacks of malarial fever every year. Port Maria, Buff Bay, Alley, May Pen, Port Antonio, Old Harbour, Savanna-la-Mar and Black River are all stations showing a high percentage of malarial attacks. Kingston shows a low malaria rate, while the higher stations in the Manchester districts are practically free.

The total number of attacks of malaria in the force in 1907 was 749, and in 1908, 820, a total for the two years of 1,569. If each attack incapacitates the man from duty for five days, which is

probably a low estimate, we obtain a total of 7,845 days lost in actual service during the two years.

### *Spleen Rate.*

These various figures, the death-rate from malaria, and the Hospital admission rate, give of course only an approximate idea of the prevalence of malaria in a community, that is to say, of the number of infected persons in a locality. But it is obvious that a very large number of individuals will not be included in statistics derived from such sources. A great many people suffer from malaria and recover, and a large proportion of these receive no hospital treatment. It is evident, then, that the percentage of infected people must be very much larger than has been shown. The only absolutely accurate method of determining the exact proportion of infected individuals in the general population would be to examine the blood of a very large number of people, taken at random from the general population, for malarial parasites, and this would give us what has been termed by Christopher and Stephens the 'endemic index' of malaria. Such a process of microscopic examination is, however, a very laborious one, and occupies a very large amount of time, as parasites are frequently very difficult to detect, and consequently, this method is one which it is rarely possible to adopt.

But there is a method which gives a very fair indication of malaria in a locality, and which was first put in practice by Professor Ross in a comprehensive manner in connection with his recent visit to Mauritius. This depends upon the fact that malaria causes an enlargement of the spleen, and consequently the number of people with enlarged spleens—what Professor Ross calls the 'spleen rate'—is a fairly reliable index of the amount of malaria in a locality, for apart from Kala-azar, which so far as I know is non-existent in Jamaica, malaria is the only endemic disease which causes a chronic enlargement of the spleen.

And it has been proved that in a locality where malaria is endemic, children up to the age of fifteen or sixteen suffer to a large extent from enlarged spleen, while over that age it appears to diminish in size, and is less easily detected. In other words, the native, as he grows older, appears to acquire a certain degree of

immunity from malaria. For our purpose, therefore, it is only necessary to examine children under that age, and this method undoubtedly affords a very valuable index for comparative purposes of the prevalence of malaria in different localities.

Following the lines laid down by Professor Ross, I endeavoured to carry out a 'splenic census' as extensively as possible in different parts of the Island. Unfortunately, at the time of my visit, the schools, which afford the best means of examining a large number of children, were closed for a month owing to the Christmas holidays, and I found it very difficult to get together a considerable number. However, with the kind assistance of the District Medical Officers, and of the Clergy, I was able to examine personally 2,036 children, and the interesting results are shown in Table X. In addition to obtaining the total number with enlarged spleens, I was at some trouble to obtain exactly the degree of enlargement and have, as suggested by Professor Ross, divided the spleens into four groups: normal spleens, and those showing three, six and nine inches degree of enlargement respectively. This gives what Ross calls the *Average Spleen*, and, as he points out, is more likely to give a more delicate index of the amount of malaria in a given locality than the simple spleen rate.

I am unable to obtain from the Registrar-General's returns any idea of the total number of children in the Island up to the age of 16, but in any case, with such a small proportion as 2,036 it is manifest that it is impossible to draw any sweeping generalisation, nor is it possible to make any comparison with the parochial death-rate, because the latter is calculated for the whole parish, while my observations were confined to small areas. But the Table is of very considerable value in this respect, that it enables us to place our finger with absolute certainty on certain spots where the endemic index is very high, and it enables us with equal certainty to exclude certain localities from the malarial area, and is of importance as indicating the localities to which anti-malarial measures should be applied without delay, and as excluding others where the urgency is not so extreme.

The following table gives the results in the different parishes. Of the children examined, over a fourth showed enlargement of the spleen.



Table showing spleen rate in various parishes

Parish	No. of children examined	Spleens				Total No. of enlarged spleens	Spleen rate	Average spleen
		1	3	6	9			
Portland .....	291	112	152	26	1	179	61.5	2.5
St. Mary .....	398	216	158	22	2	182	45.7	2.1
St. Thomas .....	44	27	10	6	1	17	38.5	2.3
St. Catherine .....	212	156	48	7	1	56	26.4	1.6
St. Elizabeth .....	249	195	47	7	0	54	21.2	1.5
Kingston .....	220	197	23	0	0	23	10.4	1.2
Westmoreland .....	278	255	17	5	1	23	8.2	1.2
Trelawny .....	189	187	2	0	0	2	1.06	1.03
St. Ann .....	69	69	0	0	0	0	0	1.0
Manchester.....	42	42	0	0	0	0	0	1.0
Chapelton .....	44	44	0	0	0	0	0	1.0
Total .....	2,036	1,500	457	73	6	536	26.3	1.2

In the St. Thomas Parish, time did not permit of my paying more than a flying visit, for which I was indebted to Dr. Edwards. At Albion, where I was only able to collect ten children, chiefly coolies, the percentage of enlarged spleens was 80, while at Yallas it was only 28.4 per cent. I have no doubt whatever that at Morant Bay and other coast villages the spleen rate would be found to be very high.

In the Portland Parish I was able, through the courtesy of Mr. Plant, the head master, to examine a considerable number of children at the Titchfield School, and though no doubt many of these came from the poorer classes, I was very much impressed with the intelligence, the good physical condition and, on the whole, the cleanliness and the evident care bestowed on the children by the parents; but even under these conditions the spleen rate was found to be from 53 to 56 per cent., diminishing as the children got older. It was interesting to note that the children residing on the ridge on which the Hospital and principal houses are built showed an almost entire absence of enlarged spleens, indicating a comparative freedom from malaria. In the lower parts of the town, however, for example, the East end and the Bound Brook side, both low-lying, badly drained and swampy, the percentage rose as high as 81.8. Here, whole families were found to be anaemic, cachectic and saturated with malaria. It ought to be noted that in this locality the examinations



were almost entirely among negro children, showing their very marked susceptibility at an early age, and it was also found that the tendency to enlargement of the spleen was greater among coloured children than among those of purer negro blood.

On the banana estates, where unfortunately the number of children examined was smaller, the percentage was very high, in two instances reaching 100. This was among the coolie population.

In this parish the average size of the spleen was two and a half times the normal.

In the parish of St. Mary, where, with the assistance of the District Medical Officers, Drs. Ritchie and Farquharson, I was able to cover a very considerable area, the spleen rates were also found to be high, an average of 45·7 per cent. Annotto Bay showed a spleen rate of 69·8 per cent., falling to 26 per cent. at Enfield, which is some two or three hundred feet above sea level.

The influence of altitude in diminishing malaria was shown at Brown's Town, where no enlarged spleen was detected. The same was found to be the case at Mandeville, and at Bethelton, both at very considerable elevations. Chapelton also showed no enlarged spleens, though there were some cases of malaria in the hospital.

This method of examination brings out the marked differences there may be in localities which are contiguous. For example, at Great Pedro Bay the percentage of enlarged spleens was 54 per cent., whereas at Newell, a short distance off, but 220 feet above sea level, it was only 2·9 per cent. This was clearly due to the character of the soil affecting the breeding places of mosquitoes. At Great Pedro Bay there were numerous grass-grown ponds in which *Anopheline* larvae could be found, whereas at Newell the country was almost entirely waterless, the soil being sandy and well drained, and the water supply derived from a few deep wells.

St. Catherine showed similar variations. Salt Pond, swampy, and situated in the midst of the banana plantations, artificially irrigated, showed a percentage of 69·2, while Spanish Town, long with a very unenviable reputation, but now with improved surface drains, showed only 17·3 per cent.

In the Black River District, one sugar estate where artificial irrigation was employed, and where a number of coolies lived in the

'rice pieces,' gave 75 per cent., while another under different conditions showed no enlarged spleens.

In Kingston, among the 220 children whom I was able to examine, there was only a percentage of 10·4. A complete splenic census of the towns would, however, show considerable variations.

It is evident, then, that in this method we have one of considerable value in determining the distribution and prevalence of malaria; and I would suggest the advisability of undertaking a more comprehensive splenic census, by having all the schools of the Colony systematically examined. The examination is a very simple one, and will require only a few seconds for each child. Two points only need be noted: the presence of enlarged spleen and the size. The age, sex, and race of child can be supplied by the teacher. The importance of this information will be evident when I discuss one of the methods of prophylaxis.

I should like to mention a point here which struck me very much in examining the schools, and that is, that the school registers in most cases showed the most marked variations in attendance, the number of absentees rising enormously at certain periods. This must inevitably interfere very much with educational progress, and tend to lower the standard, and as a considerable proportion of these absences are undoubtedly due to malaria, any diminution in that disease among the children which would promote more regular attendance would indirectly foster the cause of education.

#### SUMMARY

I may now summarise the facts which we have ascertained regarding the prevalence of malaria:—

1. The total malarial deaths for the whole Island during ten years amounts to 34,695.
2. This is equivalent to an average annual death-rate of 4·4 per thousand.
3. The average percentage of malarial deaths to total deaths is 19·7, representing nearly one-fifth of the total deaths.
4. The total admissions to hospitals from *all causes* has risen from 16,103 to 21,837 in four years, an increase of 26 per cent.

5. The total admissions from malaria have risen from 4,827 to 7,510, an increase of 55 per cent.

6. Over 33 per cent. of the total admissions were due to malaria.

7. The annual cost to the Colony of treating malarial patients in hospital is over £6,300.

8. The annual loss of labour from malaria among indentured coolies on certain estates amounted to 16'9 out of every 100 working days.

9. Among the Constabulary, the loss of working days from malaria in two years amounted to 7,845.

10. The Average Spleen Rate among the children examined was 26'3 per cent., or over a fourth with enlarged spleens.

11. The Average Spleen was 1'2.

12. There is a large interference with education on account of illness, which is preventible.

### VIII. ANTI-MALARIAL MEASURES ELSEWHERE

Before embarking on measures which are designed to reduce the amount of Malaria, and which will undoubtedly involve the expenditure of public money, no doubt the practical business men of the Colony, who control this expenditure, will require some assurance that such measures are likely to be beneficial, and it will therefore be advisable to describe briefly the steps which have been carried out elsewhere, and to indicate the measure of success which has been attained.

#### I. ISMAILIA

The town of Ismailia is situated on the Suez Canal in the midst of a desert, and is supplied with fresh water by means of a canal brought from the Nile, which was constructed in 1877. Immediately after this malaria appeared, and gradually increased in amount, the maximum being reached in 1891. In 1902 Professor Ronald Ross was called in to advise, and as a result, a vigorous anti-mosquito campaign was initiated, the marshes being filled up with sand, the irrigation channels deepened or treated with oil, mosquito nets were more generally employed, and quinine prophylaxis was had recourse to. The results were immediate and striking, as is shown in the table given below. The number of cases of malaria, which in 1902 was 1551, fell in the following year to 214, and in 1905 to 37, while in 1907 the Canal Company officially reported that 'all trace of malaria has disappeared from Ismailia.' Mosquitoes have not been entirely abolished, but they have been very greatly diminished in number.

Years	Cases Malaria	Years	Cases Malaria
1886	2,300	1896	1,150
1887	1,800	1897	2,089
1888	1,400	1898	1,545
1889	1,450	1899	1,784
1890	1,900	1900	2,284
1891	2,500	1901	1,990
1892	2,050	1902	1,551
1893	1,750	1903	214
1894	1,100	1904	90
1895	1,350	1905	37

Anti-  
malarial  
measures



Of course it must be admitted that the conditions at Ismailia were extremely favourable, the area of land to be dealt with was limited, and the conditions very easy, but the results demonstrate very clearly the *immediate* effects of systematic anti-malarial measures.

## 2. FEDERATED MALAY STATES

In the Federated Malay States at Klang and Port Swettenham, very satisfactory results have been obtained. Here the measures adopted were extensive drainage of swamps, mechanical prophylaxis, that is, making the houses mosquito-proof by means of wire gauze, and quinine distribution.

The following tables give some of the results:—

### 1. *Cases of malaria admitted to Klang Hospital from the two towns, compared with those admitted from the district.*

	1901	Anti-malarial measures.			
		1902	1903	1904	1905
Towns of Klang and Port Swettenham...	610	199	69	32	23
District .....	197	204	150	266	353

There is thus a fall from 610 in 1901, when the anti-malarial campaign was instituted, to 23 in 1905, while an increase took place among those from outside.

### 2. *Deaths in Klang and Port Swettenham.*

	1900	1901	1902	1903	1904	1905
Fever .....	259	368	59	46	48	45
Other causes .....	215	214	85	69	74	68

### 3. *Deaths in District excluding Towns.*

No anti-malarial measures.						
	1900	1901	1902	1903	1904	1905
Fever .....	173	266	227	230	286	351
Other diseases .....	133	150	176	198	204	271

*Sick Certificates and sick leave granted to Government employés:*  
(Number 176 in 1901 and 281 in 1904).

	1901	Anti-malarial measures.			
		1902	1903	1904	1905
Certificates .....	236	40	23	14	4
Days of leave .....	1,026	198	73	71	30

Dr. Watson, the Medical Officer, makes an important remark regarding the reduction of mosquitoes:—‘A definite improvement in the health of Klang was evident *when only the swamps nearest to the main groups of houses had been dealt with*, and while other swamps within the town were still untouched. The mosquitoes from these did not appear to travel any distance, and there has been no evidence of dangerous immigration of Anophelines from the extensive breeding places which, until the middle of 1904, existed just outside the town boundary, and some of which still remain. Yet the species breeding in those swamps were identical with those breeding in the town.’

### 3. HONG KONG

In Hong Kong an anti-malarial campaign, drainage, wire gauze, oiling the pools and quinine prophylaxis, was started by Dr. Thompson in 1901, and here it must be remembered that owing to the constant daily migration of 3,000 to 6,000 natives from the country districts, the difficulties of stamping out malaria are much greater, as many of these must remain infected in spite of local measures. But in spite of this the malaria reduction is very striking.

Malaria statistics in two large hospitals.

							Anti-malarial measures			
	1896	1897	1898	1899	1900	1901	1902	1903	1904	1905
Admissions .....	—	1,021	865	780	1,220	1,294	752	568	433	419
Deaths .....	—	197	126	63	163	132	128	63	58	54

Admission rate of Police for Malaria.

Admissions per cent.	32	25	19	31	42	44	19	18	11	12
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Deaths from Malaria.

Population .....	239,419	—	—	—	—	—	—	—	—	377,850
Total deaths.....	533	554	530	546	555	574	425	300	301	285
Deaths in city (Chinese only)	290	302	280	218	242	281	189	152	90	87

#### 4. SIERRA LEONE

Here, as the difficulties of immediately dealing with the town were great, the principle of segregation was adopted. A cantonment was constructed on the hills away from native dwellings, with the result that malaria has entirely disappeared among the resident European Government officials. Among Government officials elsewhere, quinine prophylaxis has been carried out with beneficial results.

In the city of Freetown, an intensely malarial place, the three main streams which were prolific sources of Anophelines have been canalised, resulting in a marked diminution in the number of mosquitoes. Oiling of the pools was also extensively resorted to, and a clause rendering it a punishable offence to have mosquito larvae in the compounds was passed. Unfortunately it is very difficult to get the native municipality, who control local sanitation, to move in the matter, and progress is very slow.

#### 5. ITALY

Some parts of this country are among the most malarious in the world, as many as 11,000 deaths formerly taking place in the year, and the difficulties were very great owing to the enormous tracts of country to be dealt with, and the nature of the agricultural cultivation. Thus, though drainage and agrarian sanitation were carried out, it was found difficult to proceed rapidly with this on a large scale, owing to the great expense involved. But while it was recognised that the ultimate aim must be the reduction of the Anopheline Mosquito by means of agrarian sanitation, it was found that an incalculable saving in health and life could in the meantime be effected by other means, namely, firstly, by methods of *mechanical prophylaxis*, that is by protecting the individual from the bite of mosquitoes, and, secondly, by *medical prophylaxis*, that is by the preventive use of quinine, and it is in Italy that perhaps this method has been most extensively and thoroughly carried out.

The following table shows the effect of *mechanical* prophylaxis alone, which was carried out along the Italian railways: the first column showing the percentage of fever attacks among people protected, the second among those not protected. The difference is striking:—

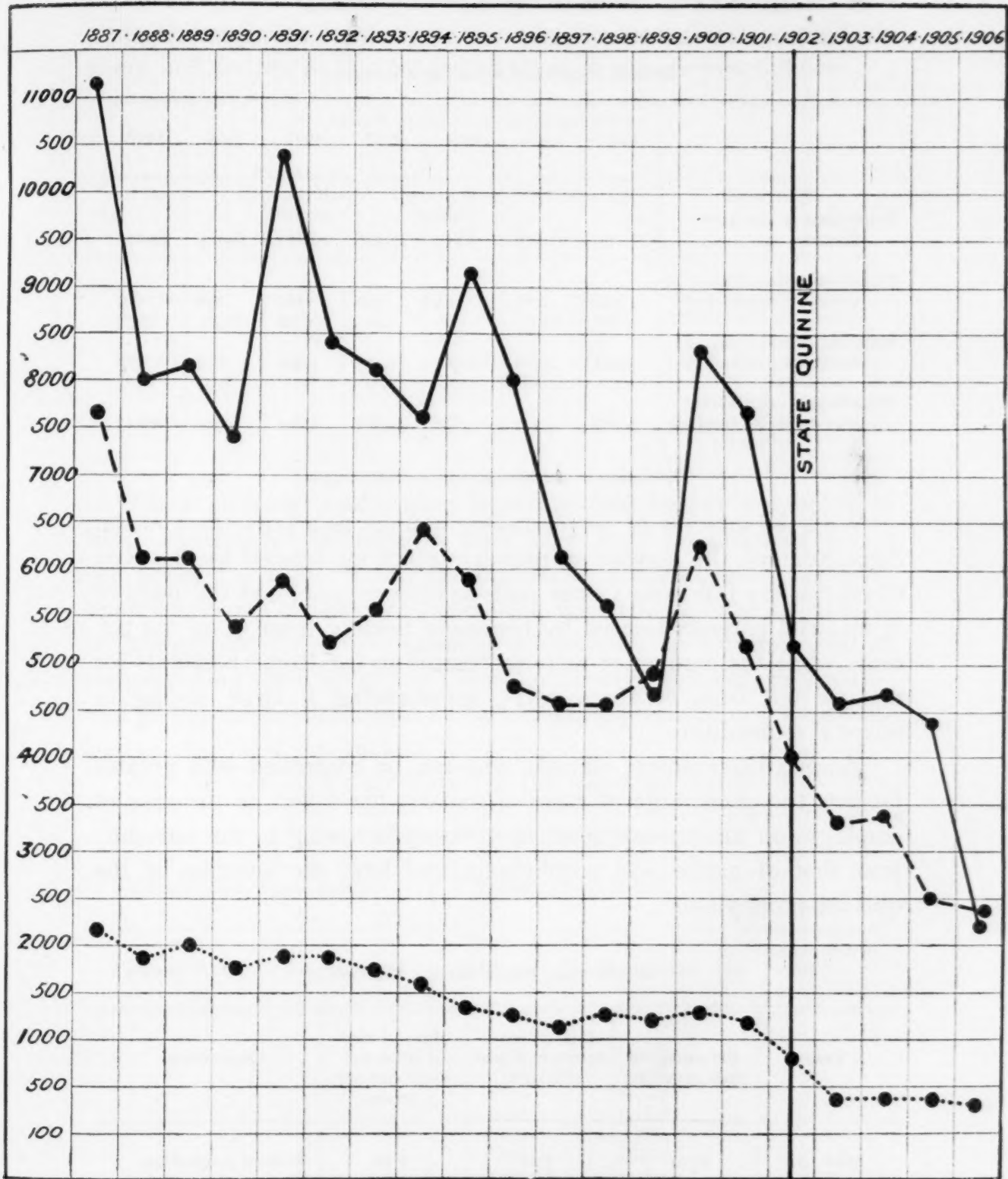
Mechanical Prophylaxis along the Italian Railways					
Year	Persons protected	Percentage attacked with fever		Percentage of control attacked	
		Recurrent	Primary	Min.	Max.
1899	24	20.0	20.0	96	—
1900	27	5.5	7.5	77	92
1901	5,165	3.3	20.2	29	96
1902	5,851	10.1	2.0	12	81
1903	8,230	22.5	4.6	10	32
1904	12,378	8.7	2.0	10	27

But clearly, mechanical prophylaxis was applicable only to a limited number on account of the expense, and hence it was found that in Italy the method which was capable of most general application and most efficient was the *distribution of quinine*, and here it is undertaken entirely by the State, distributed gratuitously to those who are too poor to pay for it, and sold at cost price to those in a better social position.

The following diagram shows the mortality from malaria in Italy before and after the commencement of State distribution of quinine.

It will be observed that the mortality from malaria is subject to periodical oscillations, with a maximum every five or six years, but that on only one occasion, from 1887 to 1902 (the year when quinine distribution was begun), in Latium and South Italy alone did the mortality sink below 5,000, while in two years it was over 10,000. *After* 1902, however, it never rose as high as 5,000, and in 1906 was under 2,500, an enormous annual saving of human life, and, further, the periodic rise which was due in 1905 or 1906 did not occur.





Mortality from Malaria in Italy before and after State distribution of quinine.

- Latium and South Italy.  
 - - - - - Sicily and Sardinia.  
 ..... Central Italy (excluding Latium and North Italy).

A similar result is shown in the following table:—

Quinine Prophylaxis in the Agro Romano							
	1900	1901	1902	1903	1904	1905	1906
Prophylaxis in the Agro Romano .....	79	1,176	3,853	17,506	29,693	38,429	41,072
Primary infections treated by the Red Cross Society	1,716 (17)	1,263 (13)	764 (7)	320 (2)	162 (1·34)	250 (1·52)	129 (0·7)
Malarial patients treated by the Red Cross Society	3,751 (31)	2,366 (26)	2,581 (20)	1,547 (11)	1,406 (10)	839 (5·1)	576 (3·4)
Malarial patients admitted into the Rome hospitals	6,186	4,725	2,750	2,461	2,991	3,991	2,513

The figures in parentheses are the percentages.

With the increase of people taking quinine as a preventive in the Agro Romano the number of primary infections treated by the Red Cross Society fell from 17 per cent. to 0·7 per cent., and the number of malarial patients treated by the same Society from 31 to 3·4 per cent., while the number of patients treated in the Rome hospitals for malaria fell from 6,186 to 2,513, representing a large saving in hospital expenditure.

Among Government officials, who can be controlled with greater facility, the percentage of cases of malaria has fallen, in the case of employés on the railway, from 69·92 to 19·84, owing to the introduction, first of mechanical prophylaxis, and later the addition of the quinine prophylaxis.

Malaria along the ex-Adriatic Railways

Year	Percentage of cases of malaria	Days of mean duration of cases of malaria	Mean of days of illness lost every year per person	Observations
1888-1905	69·92	7·88	5·48	Without prophylaxis
1902	44·93	6·99	3·12	Mechanical prophylaxis
1903	30·32	6·25	1·89	" "
1904	33·10	7·33	2·48	" "
1905	39·44	7·64	3·01	" "
1906	19·84	8·52	1·69	Mixed prophylaxis

Similarly among Customs House officers the percentage of cases of fever has fallen from 65.30 to 12.73 by mechanical prophylaxis alone, and further to 7.30 by the addition of quinine prophylaxis.

Malaria in the Customs House Officers

Year	Number of Customs House Officers	Cases of fever verified	Percentage	Observations
1900-1902	1,738	1,035	65.30	Without prophylaxis
1903	1,751	222	12.73	Mechanical prophylaxis
1904	1,714	209	12.19	" "
1905	1,721	187	10.86	Mechanical prophylaxis and beginning of quinine prophylaxis
1906	1,614	118	7.31	Mechanical and chemical prophylaxis

These striking results have been obtained largely by means of State legislation. A series of laws have been promulgated giving the public the right to buy quinine at a minimum price, imposing upon employers the duty of preventing the damage done by malaria by giving the preventive quinine gratuitously to workers, and of compensating them by giving them the curative quinine gratuitously, and giving the *poor* the right to have quinine given them gratuitously by the charitable institutions.

And the net results are shown in the following Table:—

State Quinine and Mortality from Malaria

Consumption of state quinine		Mortality from malaria		Net profits of administration of State quinine in lire
Financial year	Kilograms sold	Solar year	Total deaths	
—	—	1895	16,464	—
—	—	1896	14,017	—
—	—	1897	11,947	—
—	—	1898	11,378	—
—	—	1899	10,811	—
—	—	1900	15,865	—
—	—	1901	13,861	—
1902-3	2,242	1902	9,908	34,270
1903-4	7,234	1903	8,513	183,039
1904-5	14,071	1904	8,501	183,382
1905-6	18,712	1905	7,838	293,395
1906-7	20,723	1906	4,871	462,290

On this Professor Angelo Cello remarks that 'It appears that the annual consumption of State quinine has progressively increased from 2,242 to 20,723 kilogrammes (4,941 to 45,673 lbs.), and that in the respective quinquennia the mortality from malaria has progressively diminished two-thirds.

'This intimate relation between the progressive increase of the one, and the progressive diminution of the other, cannot honestly be ignored or denied.

'In fact from 1887 to the end of 1895 upwards of 15,000 persons died annually from malaria. . . . Owing to the introduction and the continuously increasing diffusion of the State quinine, the mortality from year to year has rapidly fallen to less than 5,000 victims, and the characteristic periodic recrudescences have no longer presented themselves.

'Now who can deny to-day that quinine is not the sovereign remedy, and that only those die from malaria who do not take it in time and in sufficient quantity?'

Concurrently with these measures a wide propaganda as to the causes and prevention of malaria has been made by means of the distribution of handbills, pamphlets, and by giving lectures and demonstrations. In other words, a serious effort has been made to *educate* the public as to evils of malaria and the means of mitigating the scourge.

## 6. THE PANAMA CANAL

To come a little nearer home, the work which has been done at Panama by the Americans is a striking lesson of what can be done by systematic and prolonged efforts on a scientific basis, by a strong Government, under climatic conditions which approximate very closely to those which obtain in the Island of Jamaica.

The measures adopted here have been the formation of a Mosquito Brigade which undertakes the cleaning of ditches, filling up swamps and holes, cleaning of pools, oiling of ditches, pools, etc., making of cement gutters, screening of houses, etc. No fewer than 2,674 lbs. of quinine were distributed.

The results, for which I am indebted to the courtesy of Professor



Sir R. Boyce, are given by Colonel Gorgas, the able and distinguished Medical Officer in charge of the Sanitary Department, as follows:—

Death rate among employés

Year	Force	Deaths	Rate per 1,000
1906	26,705	1,105	41.37
1907	39,343	1,132	28.77
1908	43,890	571	13.01

The death rate among the black employés has fallen as follows:—

Year	Force	Deaths	Rate per 1,000
1906	21,441	1,083	47.24
1907	28,634	953	33.28
1908	31,507	402	12.76

This means that in 1906 out of every 1,000 blacks on the rolls, 47 died, while in 1908 only 12 died, that is to say one-quarter of the deaths.

Among the total population of Panama, Colon and the Canal Zone, the deaths were as follows:—

Year	Population	Number of deaths	Rate per 1,000
1906	66,011	3,544	49.10
1907	102,133	3,435	33.63
1908	120,097	2,983	24.83

That is to say, in 1908 the death-rate was *half* what it was in 1906.

In 1906 there were 233 deaths; in 1907, 154 deaths; in 1908, 73 deaths. That is with a force more than one-third larger, there were in 1908 one-third fewer deaths from malaria than occurred in 1906.

Colonel Gorgas remarks:—‘I consider malaria the best measure of the sanitary work done. In 1906 out of every thousand employés we admitted in our hospitals from malaria, 821; in 1907, 424; in 1908, 282; that is, we now have only about one-third the amount of malaria among our employés that we had three years ago.’

## 7. HAVANA.

Although the following figures do not refer to malaria, they are of great importance as showing the effect of anti-mosquito measures, in the diminution of yellow fever, to an outbreak of which Jamaica

is at any moment liable, as there is an ample supply of the yellow fever mosquito, the *Stegomyia*, available all over the Colony.

Deaths in Havana from Yellow Fever.

Year	Deaths	Year	Deaths	Year	Deaths	Year	Deaths
1871	991	1881	485	1891	356	1901	18
1872	575	1882	729	1892	357	1902	0
1873	1,244	1883	849	1893	496	1903	0
1874	1,425	1884	511	1894	382	1904	0
1875	1,001	1885	165	1895	553		
1876	1,619	1886	167	1896	1,282		
1877	1,374	1887	532	1897	858		
1878	1,559	1888	468	1898	136		
1879	1,444	1889	303	1899	103		
1880	645	1890	308	1900	310		

Thus it will be seen that from a large annual mortality from yellow fever in the town of Havana, the mortality fell to *nil*, as the result of systematic, scientific measures directed against the mosquito alone.

But the subsequent history is still more interesting:—

In 1902 the American Governor had handed the Administration over to the Cubans and had left the Island. The sanitary administration evidently then gradually became lax, anti-mosquito measures were not carried out systematically, and in November, 1905, the first case of yellow fever was reported, though it was ascertained that two cases had occurred in October. A short epidemic followed, and by the middle of February, 1906, 72 cases with 23 deaths had occurred, and up to June 30th, 1906, 82 cases with 28 deaths.

But in 1907 the United States again took over the Government, and the following passage occurs in the Annual Report of the Public Health and Marine Hospital Service for 1907.

‘Following a resolution, the administration of Cuban affairs again devolve upon the Government of the United States. From a sanitary point of view this transfer was significant. There had been laxness in sanitary work in the interior, and many districts had retrograded to deplorably unhygienic conditions, requiring attention. Among the first undertakings of the new regime was the creation of an efficient sanitary service, supervised by an officer of the Army Medical Department. . . . The benefits have been promptly shown in the elimination of yellow fever from Havana.’

In the interior of the Island, after its re-introduction in 1905, Yellow Fever continued to spread, and this was attributed to the 'great lapse in Sanitation,' and the lack of hearty co-operation on the part of native Cubans. Consequently 'an act was promulgated in August, 1907, nationalizing the Sanitary Service, abolishing Municipal Boards, and placing public health affairs in charge of a central body, presided over by the Chief Sanitary Officer,' and no doubt in the Annual Report for 1909 the effect will be apparent.

In Havana, the result of the new Sanitary administration has been at once apparent, as shown in the following statistics:—

	Cases of Yellow Fever		Deaths	Percentage
Year ending 30th June, 1906	...	82	28	34
" " 1907	...	61	9	15
" " 1908	...	9	1	11

The necessity of continuity, system, and permanence in Sanitary administration, to which I shall draw attention later, is here exemplified in the most striking manner.

#### IX. ANTI-MALARIAL MEASURES IN JAMAICA

##### MEASURES ALREADY IN FORCE

The first question is, what steps have already been taken in the direction of the Prevention of Malaria, and an inquiry showed that these are practically *nil*. The following replies from the District Medical Officers give the results:—

Stoney Hill: 'No anti-malarial measures are taken in this district.'

Morant Bay: 'Nets used by the better classes. Some families take some care in dealing with possible breeding places for mosquitoes. No general preventive measures have been adopted.'

Hordley: 'None, so far as I am aware.'

Port Antonio: 'No anti-malarial measures taken by the Local Sanitary Authorities. Mr. Mitchell has filled in ten acres of morass privately.'

St. Ann's Bay: 'Beyond drainage, no anti-malarial measures have been taken in the district.'

Cave Valley: 'No special anti-malarial measures have been taken in this district.'

Falmouth: 'No anti-malarial measures have been taken in this district.'

Savanna-la-Mar: 'Very little care is taken by any section of the community to protect themselves from malaria. Mosquito nets are in fairly universal use.'

Mandeville: 'Fever does not exist or arise in my district except as an occasional sporadic case at Porus. (!) Therefore there are no anti-malarial measures necessary.'

Chapelton: 'No anti-malarial measures are taken.'

Kingston: 'No anti-malarial measures have been taken.'

Lucea: 'No special anti-malarial measures have been taken beyond the usual routine of quinine administration and advice to patients to use bed netting.'

Black River: 'None taken.'

Buff Bay: 'Anti-malarial measures taken in the district:—

(a) Swamps in various parts of the town and district have either been drained or filled up.

(b) Concrete drains have been laid in the principal streets.

(c) Quinine has been distributed to the indentured immigrants on all the estates in my district.'

Annotto Bay: 'The anti-malarial measures taken in the district are few and unsatisfactory. Quinine is supplied to the estates for indentured immigrants. This, however, as a prophylactic, has not been given a fair trial. The use of mosquito nets is limited to a very small percentage of the population.'

#### PREVENTIVE MEASURES RECOMMENDED

##### *Preliminary Observations.—Financial*

Before considering the practical measures of anti-malarial sanitation, it is advisable to make one or two preliminary observations.

And first of all, it would be useless making recommendations which are clearly beyond the financial capabilities of the Colony; all



measures must be limited to the amount of money which is available. It would be futile, for example, to advise extensive engineering schemes which, though theoretically correct and advisable, would involve the immediate expenditure of thousands of pounds, or at any rate, of amounts which could not be obtained without crippling the financial resources of the Colony for many years, and might not be obtainable at all. Obviously then our aim must be a compromise between the demands of theory and the exigencies of actual circumstances, and the results attained in Italy afford a very good example of what may be done by keeping this in mind.

### *Agricultural*

Similarly, as malaria has been shown to be dependent to some extent in this Colony upon the agricultural methods, it would be useless to suggest measures which would involve hampering or perhaps putting a stop to the agricultural development which is at present such a striking feature of some parts of Jamaica. If the eradication of malaria means the giving up of banana plantations, for example, or of imposing too great a burden on them, I am afraid that, not only the planters but the public generally, will shrug their shoulders and say, 'Well, we must put up with the malaria, we must recognise a certain amount of inefficiency from sickness, and we must provide for a certain toll on human life.' The progress of Jamaica is dependent upon its agricultural development, and the methods adopted must assist, not interfere, with this. And if I can show that, by slight modifications of these methods, by the adoption of comparatively simple precautions, necessitating, no doubt, thought, supervision and perseverance, a very great improvement can be effected, I am convinced that the common sense and business-like qualities of the estate owners will lead them to adopt the necessary measures, and I think the Government will be justified in calling upon them to do so.

### *Government*

Then it must be observed that all measures will fall naturally into two classes, namely, (a) those which must be undertaken by the Government, or a municipality, or a parochial board, or some organisation possessing funds at its disposal, and (b) those which are

more or less personal, and must be undertaken by the individual. Among the former would come such major works as drainage, the filling up of swamps, and the local arrangements and organisation for carrying out the measures detailed below. And it is essential that this body should form some definite plan of campaign which, once formed, would be continuous and systematic, and would not be liable to alteration by conflicting local interests or petty jealousies. Perseverance, continuity, and permanence are essential.

This will undoubtedly require legislation in certain directions, and in framing legislative measures in connection with sanitation, it is necessary that they should be as simple as possible, and as little burdened with legal technicalities and machinery as practicable.

In my experience of the Tropics, I have seen admirable measures rendered a dead letter through the difficulty of readily and easily bringing offenders within the meshes of the law, and if such measures are to be practically effective we must ask for, and I am sure we shall obtain, the whole-hearted co-operation of the magistracy.

To the share of the Government or central body will also fall the duty of collecting data with reference to malaria, receiving reports, and noting results. There must be some method of observing the effect of the different measures, and the central body will thus be able to control the expenditure, diminishing it where improvement has been effected, and increasing it where required.

#### *Personal*

As regards (b) the measures to be undertaken by individuals, this will resolve itself largely into a question of personal hygiene. As in all sanitary schemes, certain work is laid upon the individual, so it must be in anti-malarial sanitation. The individual must not be allowed to be a danger to the community by harbouring Anopheline larvae in his compound any more than he would be allowed to conceal a case of smallpox, and employers of labour must either personally, or by means of their overseers, see that the measures decided on are effectively carried out.

#### PRACTICAL MEASURES AGAINST MALARIA

Now we come at last to a consideration of the practical measures which are especially applicable to Jamaica, many of which have already been incidentally mentioned.

And these, as I have already shown, will depend upon two factors

- (a) the infected mosquito, and
- (b) the infected individual.

(a) **Mosquito reduction:** It is evident that in a well watered island like Jamaica, with large areas under cultivation, it is out of the question to hope for the complete extermination of the mosquito. But this is not necessary: a *reduction* in the number of mosquitoes is sufficient, and this can be effected with little difficulty.

I do not propose here to enter into a discussion of the factors which govern the numbers and diffusion of Anophelines in any given locality, but would refer those who wish to study this part of the subject more minutely to other scientific papers, and especially to Professor Ross's Report on Malaria in Mauritius. But it is self-evident that the further off a breeding place is from an inhabited locality, the fewer mosquitoes will reach the inhabitants of that spot. If we have two pools in the neighbourhood of a town, A at 50 yards breeding, say, 100 Anophelines, and B at 100 yards breeding the same number, if we can so treat breeding pool A that the Anophelines do not breed there, we have diminished very largely the probabilities of Anophelines reaching the inhabitants from the distant breeding pool B. So that the problem does not involve the destruction of mosquitoes over large areas, but resolves itself into their extermination or reduction in the immediate vicinity of inhabited places; and it has been shown by practical experience elsewhere (see note on Federated Malay States) that mere *reduction* is sufficient to cause a large diminution in the amount of malaria. And if to these we add a reduction in the number of infected individuals by various means, we get a still further proportionate diminution in the amount of malaria.

(1) **Rivers and Swamps.** The first step, and the most important as affording eventually a permanent solution of the problem, is of course the *drainage and filling in of swamps* in the immediate vicinity of towns. Most of the principal towns are situated at the mouth of rivers, and consequently are surrounded by swamps. But I recognise that this is a very large engineering question, and if an attempt were made to overtake it at once, would involve an expenditure of public money which is out of the question. So that



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I do not advocate any extensive attempt to do this at once. But I do suggest that each town should undertake the *gradual* filling up the swamps in its immediate neighbourhood. A special portion of the swamp should be selected and all town rubbish should be deposited in such spots.

In some places this has been done with great benefit, for example, at Port Antonio a considerable area of the swamp has been filled in to form a cricket ground, and a piece of ground, which formerly supplied thousands of mosquitoes, is now solid ground—a most excellent piece of reclamation. At Folly Point, too, by the enterprise of a private individual, Mr. Mitchell, a considerable area of ground has been reclaimed; and I understand he was anxious to do more on a piece of land which was not his own property, but was unable to obtain permission from the owner. This is regrettable, and I think that in such cases private idiosyncrasies should not be allowed to stand in the way of public improvements. At Annotto Bay, too, a gentleman, Mr. Westmoreland, is depositing his cocoa-nut refuse on a swamp at one side of the town, which will eventually have the effect of reclaiming this portion. These are examples of the object to be kept in view, constant and *systematic* filling up of the swamps, and if the supervising boards will carry this out much will be done. But it must not be haphazard, it must be *continuous*.

But while this is going on other palliative measures can be adopted. Where there is a tendency for the sea to back up the outlet of the river, an endeavour should be made to keep it open. This will reduce the area under water behind, and, if regularly done, should not involve much expenditure.

Then, I have already pointed out that the Anophelines breed in shallow water where there is grass and weeds. Therefore, *clear out the grass and weeds*, and especially at the edges. A great improvement can be effected by deepening the pools at the edges, and making a square margin with rough stones. This should not be costly near the beach, where stones are available.

Where the area of swamps is considerable, with scattered pools, a trench or two will be found beneficial. A trench collects the water and is more easily kept clean and treated than a large area.

In some instances it may be practicable to admit salt water to pools or trenches where the level is low. The possibility of this

should be remembered, as Anophelines, with very rare exceptions, do not live in salt water.

So far as rivers themselves are concerned, the only dangerous parts are the shallow grass-grown edges. These should be deepened where practicable, and in any case cleared of weeds. Of course I am referring solely to the parts in the immediate vicinity of towns and villages. The rivers and streams in country districts need not be dealt with. Where small shallow streams run through towns, they must be *canalized*, so as to confine the water to a limited area during the dry season. During the rains, when the current is strong, they are not dangerous.

But there is another method which is very effective in pools and all standing water, and which should always be adopted *in addition* to the above measures. I refer to the *oiling* of the surface of the water with crude kerosene. Larvae have to come to the surface to breathe—cover the surface with a thin layer of kerosene oil and they are suffocated. This alone will produce an enormous diminution in the number of Anophelines. It is especially applicable to Annotto Bay, where the pools are extensive and situated right in the middle of the town. Crude kerosene is cheap and it is very easily applied. A long stick with a few rags on the end, or a whitewash brush dipped in a tin of crude kerosene and then splashed on the surface of the water, will form a film over a large area, and one man could oil the whole of the pools in a single day. As the larvae take, on an average, from five to seven days to breed out, oiling twice a week would be sufficient. I am informed by Dr. McCatty, a very enthusiastic and keen advocate of anti-malarial measures, that the Parochial Board of Montego Bay have obtained a supply of crude kerosene, and that oiling the pools is to be extensively carried out.

(2) Treatment of **shallow ditches and gutters**. In towns, wherever it is possible, these should be cemented as is being done in Montego Bay, Savanna-la-Mar, and other places. A cemented gutter is easily kept clean. But this work, of course, will be gradual, and in the meantime they must be kept free from grass and weeds, and regularly oiled. The bottom should, so far as possible, be levelled so as to allow of an even flow of water.

(3) **Cattle ponds** caused by surface drainage. As a rule, these are not in the immediate vicinity of dwelling-houses, and in such



cases need not be troubled about; but when they are, they should be dealt with on similar lines. Clear away all grass and weeds, deepen the edges, and where possible form small embankments. Use kerosene oil when the water is not used as a water supply. In certain waters larval-feeding fishes, 'ticky-tickies,' &c., are to be found. These should be encouraged and protected as far as possible, but they are a less reliable defence than oil.

(4) **Accidental and temporary pools.** These should be filled up with earth or stones where possible; if not, oiled twice a week.

(5) **Wells.** These should be kept clean and free from growths round the edges, and in addition should be screened by being provided with a wire gauze cover. It should also be made compulsory to screen all barrels, tanks and other receptacles for storing water, as these breed other and harmful varieties of the mosquito.

(6) Now we come to **drainage trenches** in banana plantations, and I have already indicated the treatment. Keep them free from grass and weeds, and oil where necessary. The oil will not harm the bananas, and cleaning the trenches will benefit them. On each estate there should be at least one 'mosquito' coolie, whose sole duty it should be to oil, and to report on all mosquito-breeding places. It is very simple to teach him to recognise the haunts of the mosquito, and an energetic 'busha' will have no difficulty in knowing when the work is being thoroughly done.

(7) **Irrigation canals.** All that can be done is to keep the canals clear of weeds, to so plan them that the gradient is even, and treat outlying pools with kerosene.

#### LEGISLATION

But these public measures will be hampered and to some extent neutralised if private individuals are to be allowed to breed mosquito larvae in their compounds. I regard it, then, as imperative, that **legislation should be introduced without delay**, making it a punishable offence to have mosquito larvae in any collection of water in a compound, that is to say, this particular insanitary condition should be placed in the same category as any ordinary 'nuisance,' which at present can be dealt with by law. No doubt opposition will be



raised to this. We shall hear about the hardship to the poor native and of his inability to recognise mosquito larvae, his ignorance of the evil results which may follow, and so on, but experience has shown elsewhere that these difficulties are not met with in practice. Every native is familiar with the 'wiggler' in water, though he may not be aware that it develops into a mosquito, and when he realises that the presence of a barrel full of 'wigglers' in his yard entails a compulsory visit to the police court, and the production of a certain sum of money, we may rest assured that they will disappear like magic. And it is not as if the suggestion involved any expense: all that is required is supervision on the part of the occupier, the frequent emptying of water barrels and other vessels, the daily sweeping out of hollows in the ground, and the cleaning of gutters. It will not be contended that this places a serious burden on the householder.

And though the suggestion may be unfamiliar to Jamaica, and possibly therefore somewhat unpalatable, it is by no means a new one. I was successful some years ago, in Sierra Leone, in getting a clause included in the Sanitary Ordinance, making it a punishable offence to have mosquito larvae in a compound, and similar laws have been passed in other West Indian colonies, and are actually being put in force. Sir R. Boyce, who has just returned from visiting the other colonies, informs me that prosecutions are being daily undertaken, and that fines of no less than £2 are being inflicted, with the most beneficial results in diminishing mosquitoes.

A very appreciable diminution in mosquitoes can then be effected by simple means, the principal cost at first being (a) cost of kerosene, and (b) wages of a sufficient number of men (which need not be great) to oil and keep down the grass and weeds. I would therefore summarise the anti-mosquito measures as follows: not in the order of their importance but of their practicability:—

1. Make the harbouring of mosquito larvae in private compounds a punishable offence.
2. Keep all margins of rivers, swamps, water channels, ditches, gutters, ponds and pools free from grass and weeds.
3. Apply crude kerosene regularly to all possible breeding places of mosquitoes.

4. Cement all gutters in towns.
5. Screen all wells, tanks, barrels, etc.
6. Gradually reclaim and drain swamps in the immediate vicinity of towns.

#### PREVENTIVE MEASURES AFFECTING THE INDIVIDUAL

We can prevent the *individual* from becoming infected by protecting him from being bitten by mosquitoes already infected; and we can prevent the *mosquito* from becoming infected by placing obstacles in the way of his biting individuals already infected, and thus becoming infected in his turn. We thus diminish two sources of danger.

The measures to be adopted come under the head of Mechanical Prophylaxis, and are mainly a matter for *personal* application:—

1. **Use of the mosquito net.** As the habits of the Anopheline mosquito are mainly nocturnal, it follows that if we protect ourselves from the bites of these animals during the time we are in bed, say from ten to six, that is to say, for eight hours out of the twenty-four, we are protecting ourselves for a third of our lives, and that, at the time when we are most defenceless and liable to attack; and yet I was astonished to find that in Jamaica the use and value of the mosquito net was so little appreciated, and that its use was not carried out in a satisfactory and efficient manner. I can only recall one single instance in which it was properly used, that is, hung inside the mosquito poles, so as to permit of its being tucked underneath the mattress.

Occasionally, when I suggested the use of the mosquito net, I was met by the answer, 'Oh, there are not many mosquitoes here, and they don't touch me.' But *one* infected mosquito is sufficient to do the mischief, and surely, apart from comfort, an elementary precaution of this kind should not be neglected, especially in the hotels. I was assured in one hotel that there were no mosquitoes, but I was kept awake for some hours, until apparently all those in the room had had sufficient nourishment, when I succeeded in falling into a troubled slumber.

The use of the mosquito net, then, as a personal protection in malarious localities, is one which should *never* be neglected by those able to afford it.

But obviously this measure is one of limited application. The mass of the general native population cannot provide themselves with mosquito nets on account of the expense, nor can it be expected that the Government should do so.

2. The same consideration applies to some extent to the next method, the use of houses or rooms made **mosquito proof** by means of wire gauze. This is undoubtedly one of the most effective of the methods of mechanical prophylaxis, and has been adopted extensively in Italy, and to some extent in West Africa and other highly malarious parts of the world. It is out of the question applying it generally, but there are certain directions in which its use would be of value, and would eventually result in an economy to the Government.

#### *Police Stations*

Among these, Police Stations may be mentioned. It has already been tried at Port Henderson, but it should be universally adopted at *all* police stations where the percentage of malarial attacks among the constables is high. These will be readily seen from Table VIII: Morant Bay, Port Antonio, Buff Bay, Port Maria, Annotto Bay, Green Island, Savanna-la-Mar, Black River, Many Pen, Alley, and Old Harbour, as being the most unhealthy, might be among the first protected. The expense of wire gauze is not great, and the beneficial results in increased efficiency would be most marked.

#### *Public Hospitals*

Another series of public buildings which ought to be dealt with in a similar way in certain localities are the Public Hospitals, and I observed in the Medical Report for 1908, that provision has been made in the estimates for doing this to some extent. In Annotto Bay, for example, it is almost criminal to collect and house, in open wards, or worse still in tents, in the middle of a populous town, malarial cases from all parts of the district, with an extensive *Anopheles* breeding pool within a hundred yards. No better device for encouraging the spread of malaria can be conceived, and it is hardly fair that the inhabitants of the place, in addition to their own malaria, should have extraneous sources of infection brought to their very doors.



I observe that the experiment of screening Port Antonio Hospital was tried, but, I understand, was abandoned because the wire gauze was interfered with by the patients. But surely this is a matter for supervision and care.

I venture to think it essential that the Port Antonio Hospital, full of malarious patients (see Medical Reports), situated, as it is, on the ridge on which the hotel and the principal dwellings are built, should be thoroughly screened on account of the danger to the general public.

#### *Coolie Barracks*

A third situation in which protection by wire gauze should be seriously considered is Coolie Barracks. I am aware that it will be urged that the habits and intelligence of the average coolie will render this difficult if not impracticable, but after all, this is largely a question of custom. At first no doubt there would be damage, but with careful supervision, and as the coolie becomes accustomed to it, I am convinced that the difficulty would disappear.

Even a hundred and thirty years ago the health of employés was a matter of concern to the planters, for I came across, in the History of Jamaica, already quoted, the following pregnant remarks, which are as applicable now as then:—

‘Those whom fortune has blest with abundance should be studious  
‘to preserve the lives of their dependents whose poverty is their  
‘greatest crime. The cruelty of exposing the lives of men to sickness  
‘or death by restricting them to live in wretched hovels or in  
‘unhealthy spots needs only to be pointed out in order to be relieved.  
‘The natural generosity and benevolent disposition of the planters  
‘will immediately lead them to administer the certain remedy  
‘although it may be attended at first with some extraordinary  
‘expense to them.’

If some public-spirited employer in Jamaica will carry out the experiment thoroughly and will carefully and accurately note the result, I am sure that the striking improvement which will be effected will lead others to follow his example.

There are several minor personal matters, such as anointing the body with various oils or ointments to prevent mosquitoes biting, fumigation of rooms by special preparations, etc., but these are unreliable and not likely to be of general application so that they need not be considered in detail.



## QUININE ADMINISTRATION

3. But there is a third method of prevention as applied to the individual, which is simple, inexpensive, effective and of easy general application. I refer to the **preventive administration of quinine**.

Although the curative effect of quinine, or rather of the bark from which it is extracted, namely cinchona, has been known since the 17th century, attention has only been drawn to its extreme value as a preventive of recent years, but it was evidently well known to the old Jamaicans. 'Strangers newly arrived in such places and those who are constitutionally subject to agues should, during the sickly season, take every other night, two or three teaspoonfuls of tincture sacra or a few grains of pilula rufi, not sufficient to purge but only to keep the body pretty open, and for further prevention a wineglassful of the infusion of bark and orange peel in water, or a tablespoonful of a strong tincture of bark may be taken diluted with water occasionally in the morning before breakfast' (History of Jamaica, 1774).

As far back as 1891 I advocated in the 'Lancet' the daily use of quinine as a preventive, and with every year's residence on the West Coast of Africa I was more convinced of its efficiency, and it was very striking to observe the immunity from malarial fever which was enjoyed by those who took it regularly, as compared with those who did not.

A reference to the section dealing with Italy will show the remarkable results which have been obtained there.

It is sometimes suggested that the long continued use of quinine has a deleterious effect, but as a matter of actual experience this is not found to be the case. On the contrary, the small doses required, generally act as a tonic. Nor does practical experience support the objection that larger doses will be required during the attack of fever if one is habituated to the drug.

Two methods of quinine prophylaxis are recommended, first, the administration of fifteen grains twice a week, and, second, the daily use of five grains. In my experience, the first method, though efficacious, if regularly carried out, is unsuccessful in practice, as it is rarely adhered to. Fifteen grains of quinine will produce in most individuals unpleasant symptoms of cinchonism, headache, buzzing in

the ears, etc.; it is apt to be forgotten and taken irregularly, and is eventually given up. On the other hand, I have had no difficulty in persuading people to take the smaller daily dose and have seen no bad effects from it. The action of the quinine, of course, is its poisonous effect on the malarial parasites, and the immature stages, more especially that which is injected from the salivary glands of the mosquito, appear to be more susceptible than the adult forms, which require larger doses.

I may consider briefly the administration of quinine to different sections of the population:—

(a) *The Police*

Here we have to deal with a disciplined body, under easy control, and the problem is simple. With a force of 1,146 station officers and men in 1908 (according to the figures supplied) a daily dose of five grains would require 4,803 ounces of quinine. At a wholesale cost of 1s. an ounce this would involve an expenditure of £240 per annum approximately if given to every man—considerably less than it costs to keep the men in hospital. But it will not be necessary to administer quinine universally. Many of the stations, as shown in Table VIII, are free from malaria, so that the regular administration of quinine at those stations is not necessary, unless for limited periods, in the case of men transferred from malarious localities. Consequently the cost will be considerably below that stated.

Here, again, a systematic method must be adopted. There should be a daily morning quinine parade at which each man should be compelled, unless exempted by the Medical Officer, to swallow five grains of quinine. This should be done under the personal supervision of the sergeant-in-charge, and the District Inspector should occasionally himself superintend. A Quinine Book, ruled in columns for each day of the month, should be kept, and an entry made opposite the name of the constable. When not taken, a note should be made of the reason for non-administration.

A monthly return should be sent in to the Inspector-General showing the number of doses given, the number of men off duty from malaria, the average daily sick in hospital from malaria, and the proportion of daily sick from malaria to daily strength. This would

afford an accurate comparison between successive months and years.

In cases where men suffer from actual attacks of malaria, they should be kept for considerable periods in hospital, and treated with large doses of quinine for a lengthened time so as to kill all parasites.

Of course, the effect will not be immediate, as so many men are already infected, and recurrences will be frequent, but as these die out, and primary infections are prevented, there will be a marked diminution in the number of inefficient men from this cause.

(b) *Indentured Coolies*

Here again we have a body of men who are under definite regulations, and under a certain amount of control, and to whom the preventive administration of quinine should not present any difficulty.

This experiment has already been tried on the recommendation of the Superintending Medical Officer, but evidently it has not been given a fair trial or carried out in a systematic and thorough manner.

I observe in the report for 1907, a number of estates mentioned, with the quantities of free quinine which were issued to them, and I have calculated the amount of quinine which should have been used on one or two.

On Wentworth estate, 46 ounces of quinine were issued. On that estate in 1907 the average daily number of male coolies employed was 31, requiring a daily consumption of 155 grains at five grains per man, or an annual consumption of a little over 129 ounces. 130 ounces at 1s. would cost £6 10s., and even if the malaria could only be diminished by one-third, it would more than repay the cost to the Government.

At Trinity, with an average daily number of 14 men, eight ounces were used instead of 58.

At Low Layton, with 21 men, seven ounces instead of 87; at Amity Hall, with an average of 44 men, 39 ounces instead of 183; at Frome, with 20 men, two ounces instead of 83.

I need not labour this point, but it is quite evident that quinine could not have been regularly given, and unless this is done, it is a sheer waste of money.

A systematic method is here required also.

There should be a morning parade under the personal supervision of a 'busha' or book-keeper; a carefully kept quinine register; and



a record of all absences from work on account of malaria as already suggested. It is useless relying on native Indian overseers, at first at any rate, until they begin to appreciate the benefits, but I am sure it is not impossible to find in Jamaica keen, enthusiastic and energetic 'bushas,' who will see that this method is given a fair and thorough trial.

(c) *School Children*

I have already shown that children are the principal carriers of the malarial parasite, and that the prevalence of malarial infection can be arrived at by ascertaining the percentage of enlarged spleens. It is evident, therefore, that if we can diminish the number of infected children we largely diminish the possibilities of the general infection.

And in the schools of the Colony we can get at the children. In discussing the matter with the Superintending Inspector of Education, he was inclined to believe that the cost of cinchonising the whole school population would be prohibitive. But here, again, we can limit the application. A considerable number of schools are situated in non-malarious districts, and can be excluded, only actual cases of malarial fever in such districts when introduced, being thoroughly treated.

And even in the malarious districts I would, in the first instance at any rate, suggest the limitation of the quinine administration to children with enlarged spleens. Now, this will involve the periodic examination of the school children, and this is one of the recommendations which I have already urged upon the Government. It will then be possible to determine from time to time the number of infected children and the effect of the quinine administration.

The school-master would be supplied with this register, and it would be his duty to administer a daily dose of quinine to all infected children, noting the amount in his register, absences from school, and any reasons for non-administration. No infected child should be exempted, except on a medical certificate.

The cost will be further diminished in the cases of children by the fact that a smaller dose of quinine is required. The daily dose may be approximately as follows:—Up to three years, 1 grain; three to six, 2 grains; six to eight, 3 grains; eight to twelve, 4



grains; and over twelve, 5 grains. In certain cases of immature or undersized children, the dose would have to be lessened, and this would be determined by the Medical Officer. It is a question rather of bulk than of age.

In the case of coolie children not attending school, the administration would be supervised by the 'busha' at the same time as the morning administration to the adult male and female coolies.

As to the form, it is probable that chocolate comfits as issued by the Italian Government will be found most palatable and most readily taken by children.

#### (d) *General Population*

Here the question is more difficult. We cannot force them to take it but we can place them in the way of getting it readily, and experience elsewhere has shown, that the lower classes very soon learn to appreciate the beneficial results of the quinine, and apply for it freely.

There are three points which experience has taught must be insisted on:—

- 1st. That it should be given **gratuitously**;
- 2nd. That it must be brought **directly to the notice** of the people;
- 3rd. It must be given in **sufficient** quantity.

1. With reference to the first, I quite expect that the objection of 'pauperising' the people will be raised. But the people are already pauperised by malaria. No man (nor woman) can work well with his blood thinned by malaria, and an anaemic pigment-clogged brain. I visited a large number of the peasant dwellings of Jamaica, and it seemed to me that there was a very large amount of real poverty in the Island. Though the people might have enough to eat, of the plainest and most monotonous description, there appeared to be little hard cash, and this view was corroborated by clergymen and others. I must confess to having felt a great sympathy with those unfortunate people in the 'bush,' more especially the women and children, and a great admiration for the patient, cheerful, philosophic way in which they endured their illnesses. There was an uncomplaining fatalism about them which was most pathetic. They looked upon their ills

as dispensations of Providence, which they had not the means to cure, and which must therefore be endured as best they could. And if the gratuitous administration of quinine will alleviate their hardships, I can hardly believe that it will be withheld.

2. The second point is that the supply must be placed in such a position as to be easily available; it must be forced on the attention of the people. It is no use saying that quinine can be had by applying to the Dispenser at Falmouth or Annotto Bay, or any of the other Hospitals. That will serve very well for the inhabitants of the locality; but a woman with fever is not going to tramp five or ten miles for doses of quinine, or if she gets one supply, she will not trouble about the next.

And there is already an identical example of this in the Island in the case of yaws. This disease is very amenable to treatment, and medicine is supplied free. But to be effective, it must be taken continuously, and I found that one bottle would be taken, and then it would be discontinued until the individual happened to be again in the neighbourhood of the supply. Hence the effect is lost, and there is a considerable waste of public money.

3. It must be given in sufficient quantity. In a family of four, for example, father, mother and two children of eleven and seven, say, living in a malarious district, enough must be given to allow them to take 17 grains a day, and it should be carefully explained to them how they are to take it.

To give them half an ounce and to expect it to last a month would be not only useless, as a preventive, but a waste of money.

Consequently some *machinery for distribution* is necessary.

The best method is by the appointment of **Quinine Dispensers** for various malarious districts, whose duties would be to visit the various hamlets, to make house-to-house visitations to find out the people who suffer from fever, to explain the proper method of taking quinine, and to report to the Medical Officer acute attacks of malarial fever. There is no reason why they should not distribute at the same time the medicine for yaws, or for another scourge of the children of the Island, worms.

They would in the first place receive tuition as to the method of administering quinine, the recognition of cases of fever, and of enlarged spleens, and should, I think, be under the control of the

District Medical Officer to whom they would report as to work done, itinerary, cases seen, etc.

An alternative method, but less satisfactory, because it would not include house-to-house visitation, would be to place the distribution in the hands of the clergymen and the police. I am sure that the former would be only too anxious to assist in every possible way, though their time is taken up by many other important duties.

Space will not allow me to go into details as to rules and regulations for such a service, but there are one or two points which I may mention.

In the first place, Government quinine should be in such a form as to render it easily distinguishable from other quinine. If in the form of pills or tablets it may be tinted, but probably the form used in Italy, namely, the tablets of quinine made up with chocolate, would be the most serviceable.

Of course there must be certain restrictions and penalties, and the sale by dispensers or others should be entirely forbidden. But the regulations must be as simple as possible, otherwise too many restrictions will defeat the object of the service, and a certain unavoidable leakage must be allowed for.

As to the well-to-do there is no reason why they should not obtain their quinine from the same sources as they now do.

#### *Quinine during acute attacks.*

And now a word in passing as to the quinine treatment of fever. I have passed a considerable period of my life in the midst of malaria, often of the most malignant types, and if I may venture a humble word of criticism, I may say that I was much struck with the comparatively small doses of quinine administered during fever in the Island. I am certainly inclined to advocate the administration of considerable doses, 10 to 15 grains, two or even three times a day, according to severity, as being more likely to thoroughly kill off the parasites, and thus limit the period of infection, and as some stages of the parasite are more resistant than others, it is important that quinine in considerable doses should be continued for a considerable time after the febrile manifestations have ceased. Consequently in the cases of police and coolies, they should be kept in hospital for rather longer periods, or some superior officer should see that their doses



of quinine are taken in the quantities ordered by the Medical Officer. By this means recurrences will be rendered less frequent and chronic infections with enlarged spleen will disappear.

#### X. ANTI-MALARIAL ORGANISATION

I have thus gone somewhat fully into the preventive measures which experience has shown to be beneficial as regards the reduction of malaria, and have indicated those which are specially applicable to Jamaica; and the question of the machinery by which they are to be carried out must now be considered. If any plan of campaign is to be carried out persistently and systematically, some form of organisation must be provided, and this applies equally to the war against disease. Our efforts must be properly directed if they are to be effective.

But the machinery must not be complicated or expensive, and the following brief sketch indicates the lines which in my opinion should be followed.

1. First there should be a **Central Malarial Board** to sit in Kingston. It should consist of members from various localities, and the medical profession should be largely represented on it. No doubt the secretarial work of the Board could, with advantage, be done by the Medical Department.

The Board would formulate a plan of campaign, consider the works and methods to be adopted in various localities, estimate cost, consider the legislation required, and receive and collate all statistics and reports as to work done.

2. **Local Boards** to report and recommend to Central Board, and to carry out the measures decided on. Their Medical Officer should be *ex-officio* a member of the Local Board.

3. **Staff of Local Boards :—**

- (a) Medical Officer. The question as to whether this work can be carried out by the District Medical Officer will have to be considered. In some districts it would be an advantage, in others it seems to me that their time is already so much occupied with other duties that they would be unable to give the necessary supervision. The duties of the Medical Officer would be general inspection of the malarial district, general supervision of the men, medical examination



of children (splenic census), periodical visits to the schools, collection of statistics as regards malaria, and preparation of reports as to work done and results. This sounds extensive, but a good deal of the work is overlapping.

(b) Mosquito gangs: These would vary in size according to locality, and would be controlled by a headman of intelligence, of whom there is no lack in Jamaica. It is important that they should have a distinctive badge, and that their authority should be upheld and respected.

Their duties would be principally:—

1. Clearing pools and swamps of weeds.
2. Deepening shallow pools and swamps where feasible.
3. Filling up depressions.
4. Oiling collections of water.
5. Inspecting yards.
6. Serving summonses for contraventions.

(c) Quinine Dispenser: Duties already detailed.

## XI. COST

I have not the material at my disposal to give an estimate of the cost of the measures which I have recommended, but I am sure that it will not be found to be prohibitive, and it has this great recommendation, that as malaria diminishes, though some of them must be permanent, others will become unnecessary, and expenditure will diminish. If we are to judge of the results elsewhere, a systematic campaign against malaria will in *three* years effect such a large reduction that there will be an enormous saving to the Government in hospital treatment alone.

## XII. EDUCATION IN SANITATION AND HYGIENE

Lastly, there is a matter to which it is necessary that attention should be drawn, and that is Sanitary Education. Adults should be attacked by means of pamphlets and circulars drawing attention to the evils of Malaria and the simple methods of its prevention, and the vast influence of the clergy can be utilised to no better purpose than by preaching at all seasons the gospel of cleanliness and sanitary surroundings. I make a strong appeal to them for their assistance.

Adults, however, are sometimes difficult to move, their ideas are more or less fixed, and their convictions too settled.

But *get hold of the children.*

I was much struck by seeing in the schools a series of practical 'Don'ts' dealing with every-day life, which was introduced by His Grace the Archbishop. Add to this some Sanitary 'Don'ts and Do's.' Hammer into their infantile heads the simple facts, 'Don't have wrigglers in your yard,' 'Do get rid of Mosquitoes,' until they become part and parcel of their intelligence, and they will never forget the lessons thus learned.

The children of this generation are the adults of the next, and if we can now instil into their minds, at its most receptive stage of growth, the great truths of Sanitary Science, we shall have, within a few years, thousands of intelligent adults, who will appreciate and be fully prepared to carry out, for their own sakes, the necessary measures to make Jamaica what, with its exquisite climate and enormous natural advantages, it ought to be, a Sanitary Paradise.

# XIII. SUMMARY OF RECOMMENDATIONS

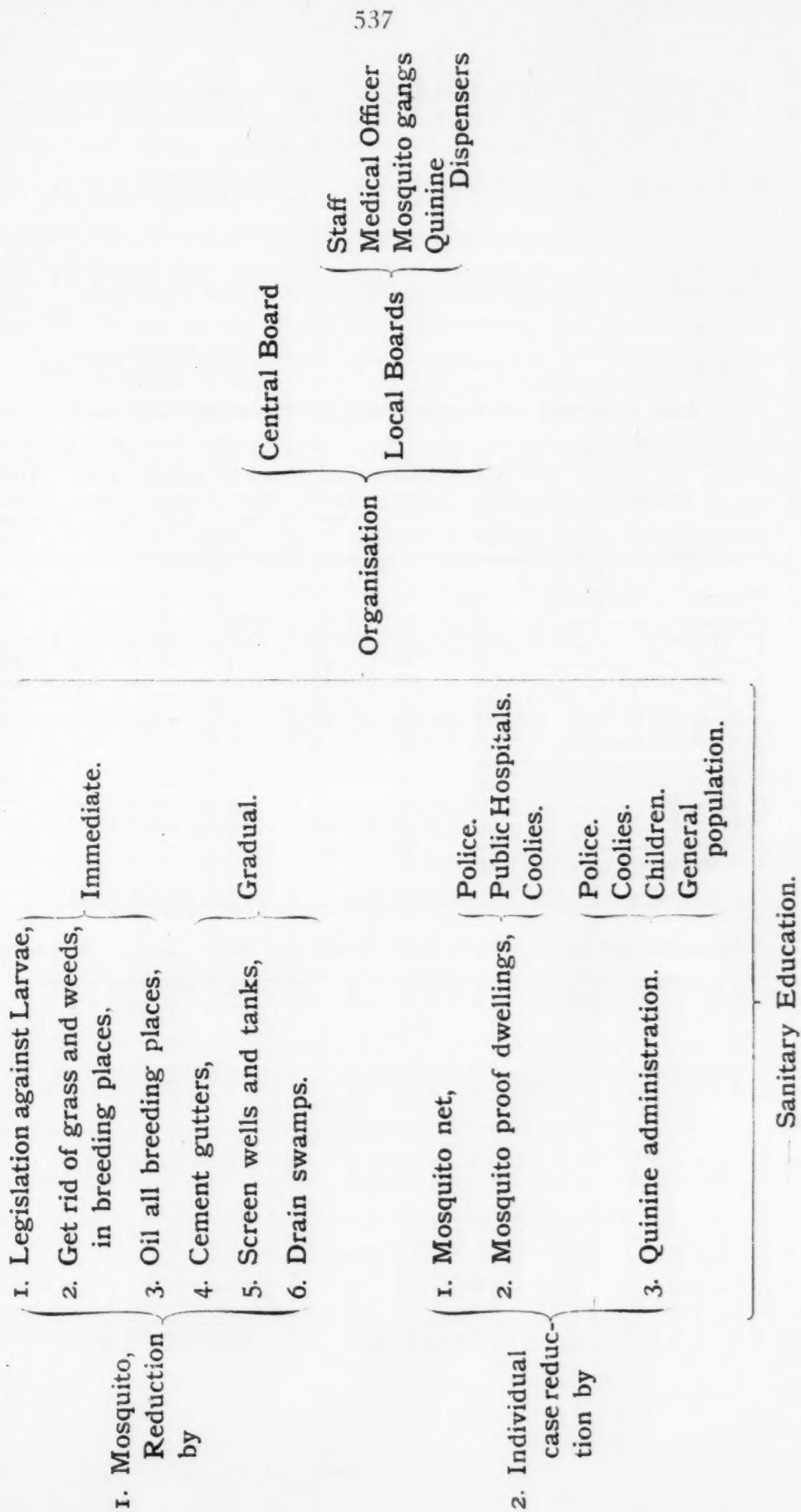


TABLE 1. Showing areas in square miles at different elevations in each parish

Parishes	Area below 1,000 feet	1,000 to 2,000 feet	2,000 to 3,000 feet	3,000 to 4,000 feet	4,000 to 5,000 feet	5,000 feet and over	Total areas in square miles
Kingston .....	6½	1½	—	—	—	—	7½
St. Andrew .....	59	54	27	17½	8	½	166
St. Thomas .....	135	59	35	20	14	11	274
Portland .....	94	89	40	32½	17	12½	285
St. Mary .....	110	116	19	4	—	—	249
St. Ann .....	85	337	54	—	—	—	476
Trelawny .....	166	135	32	—	—	—	333
St. James .....	139	90	5	—	—	—	234
Hanover .....	161	6	—	—	—	—	167
Westmoreland .....	235	73	—	—	—	—	308
St. Elizabeth .....	335	120	7	—	—	—	462
Manchester .....	42	134	126	—	—	—	302
Clarendon .....	314	115	45	—	—	—	474
St. Catherine .....	336	124	10	—	—	—	470
Totals .....	2,217½	1,452	400	74	39	24	4,207½



TABLE 2. The Island Monthly Rainfall from 1880 to 1905

Year	Jan.	Feb.	Mar.	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Totals
1880.....	in. 4.36	in. 0.96	in. 1.10	in. 2.77	in. 11.60	in. 3.09	in. 3.86	in. 9.58	in. 3.97	in. 4.00	in. 2.21	in. 7.94	in. 55.44
1881.....	1.22	4.01	1.30	4.63	10.28	5.56	4.77	6.21	7.68	12.08	7.52	3.34	68.60
1882.....	2.92	1.93	3.54	3.32	2.33	2.33	3.76	4.80	8.78	8.96	5.36	3.95	57.87
1883.....	5.49	3.50	4.08	3.34	5.29	4.98	3.15	5.42	7.82	8.15	5.12	2.92	59.26
1884.....	4.72	3.44	2.51	1.85	6.72	6.89	2.52	5.06	6.23	9.52	5.00	2.44	56.90
1885.....	1.73	1.49	1.47	4.73	4.90	3.32	3.01	6.19	6.22	6.37	4.74	15.69	59.86
1886.....	5.23	4.65	2.68	6.39	5.30	23.36	6.22	13.54	5.90	7.98	3.70	5.66	90.61
1887.....	6.02	2.32	2.38	4.47	9.32	8.89	7.9	6.91	5.77	8.47	8.17	0.75	70.66
1888.....	1.36	1.89	1.70	3.61	21.24	6.77	2.65	5.47	8.10	4.38	4.59	10.35	72.11
1889.....	4.78	0.90	4.19	6.71	7.82	12.52	6.08	5.12	8.20	10.49	4.37	2.97	74.15
Means .....	3.78	2.51	2.49	4.18	9.07	7.77	4.32	6.83	6.87	8.04	5.08	5.60	66.54
1890.....	5.21	2.92	5.84	3.37	5.57	4.13	4.99	6.92	6.52	7.04	6.52	5.39	64.42
1891.....	3.45	2.24	0.84	8.49	12.28	9.91	5.57	7.45	6.35	15.32	7.65	5.15	84.70
1892.....	4.00	1.38	2.27	2.82	8.53	7.31	4.44	7.65	8.86	12.17	9.96	3.61	73.00
1893.....	3.44	3.24	1.92	5.42	10.90	7.20	9.15	6.72	7.92	10.30	10.10	10.18	86.49
1894.....	2.05	2.52	3.33	5.84	16.64	3.90	5.92	4.20	6.98	12.40	5.05	6.56	75.39
1895.....	1.31	5.00	2.18	6.11	9.90	3.66	4.99	8.11	6.87	11.98	7.72	3.79	71.62
1896.....	5.25	4.86	4.28	3.67	9.96	4.84	5.03	4.74	8.24	7.51	4.57	5.66	68.61
1897.....	0.88	0.77	1.82	7.06	10.91	4.92	5.92	6.55	10.13	19.26	5.73	3.64	77.59
1898.....	1.75	3.93	1.26	4.09	16.76	7.60	6.50	6.92	7.10	10.38	4.78	2.75	73.82
1899.....	3.96	2.84	3.76	4.80	4.20	4.66	3.86	4.22	7.44	23.72	14.99	7.37	85.82
Means .....	3.13	2.97	2.75	5.17	10.56	5.81	5.64	6.35	7.64	13.01	7.71	5.41	76.15
1900.....	5.20	4.15	2.42	5.67	7.77	6.16	7.18	5.38	8.12	6.50	5.22	5.88	69.65
1901.....	3.91	1.17	3.32	2.57	6.13	14.03	7.59	6.49	10.60	9.76	10.02	5.37	80.96
1902.....	5.68	3.06	4.24	5.40	8.97	10.28	3.44	5.39	5.89	7.19	5.60	8.23	73.37
1903.....	1.94	1.40	3.19	4.90	10.63	6.00	4.30	12.79	5.34	7.28	5.78	4.83	68.38
1904.....	3.42	4.66	6.84	5.91	7.51	15.20	4.26	5.47	6.49	16.58	7.87	3.94	88.15
1905.....	7.83	2.99	7.48	5.14	8.20	10.10	2.73	6.7	8.27	12.36	6.77	7.17	85.20
1906.....	3.37	5.15	5.50	8.02	13.23	11.47	4.19	6.98	10.70	8.44	7.60	2.06	86.71

TABLE 3. Annual Rainfall for each Rainfall Division in Jamaica

Year	RAINFALL DIVISIONS				The Island
	N.E. Division	N. Division	W.C. Division	S. Division	
	in.	in.	in.	in.	in.
1870.....	110.60	83.09	102.98	61.07	89.43
1871.....	69.45	41.88	54.56	34.46	50.09
1872.....	59.42	40.79	51.50	29.02	45.18
1873.....	84.08	52.64	67.79	47.71	63.06
1874.....	97.18	68.25	62.97	47.35	68.94
1875.....	71.89	47.15	56.16	34.47	52.42
1876.....	90.38	54.71	87.33	52.99	71.35
1877.....	100.72	56.53	64.06	52.27	68.40
1878.....	104.12	62.99	72.44	66.11	76.42
1879.....	122.55	65.44	87.54	79.85	88.84
Means .....	91.04	57.34	70.73	50.53	67.41
1880.....	76.37	47.01	64.91	33.47	55.44
1881.....	91.24	49.42	75.32	58.42	68.60
1882.....	65.48	43.76	78.59	43.67	57.87
1883.....	72.30	41.52	78.19	45.02	59.26
1884.....	69.00	41.87	73.10	43.63	56.90
1885.....	70.55	52.77	72.62	43.52	59.86
1886.....	126.61	60.98	88.21	86.64	90.61
1887.....	80.25	61.07	80.14	61.16	70.66
1888.....	98.00	54.42	70.43	65.58	72.11
1889.....	99.81	56.82	75.94	64.02	74.15
Means .....	84.96	50.96	75.74	54.51	66.54
1890.....	75.09	48.29	89.91	44.41	64.42
1891.....	110.56	66.71	100.50	61.03	84.70
1892.....	101.55	58.10	82.05	50.29	73.00
1893.....	106.50	63.17	108.66	67.65	86.49
1894.....	90.56	54.04	95.93	61.01	75.39
1895.....	97.38	56.35	85.38	47.36	71.62
1896.....	95.42	54.90	78.31	45.79	68.61
1897.....	93.95	58.25	95.46	62.67	77.59
1898.....	102.92	52.44	84.26	55.67	73.82
1899.....	112.10	61.31	101.28	68.62	85.82
Means .....	98.60	57.36	92.17	56.45	76.15
1900.....	96.91	50.67	79.84	51.15	69.65
1901.....	107.88	64.18	87.31	64.50	80.96
1902.....	95.97	58.78	89.75	49.14	73.37
1903.....	88.46	51.05	82.83	51.17	68.38
1904.....	112.12	63.72	104.40	72.35	88.15
1905.....	112.91	61.33	94.23	72.31	85.20
1906.....	109.69	56.25	100.90	79.96	86.71

TABLE 4. Average Annual Temperatures at different elevations in Jamaica

Elevation above sea-level	Mean	Max.	Min.	Range
Feet				
0	78.8	87.5	70.8	16.7
500	77.1	85.1	69.8	15.3
1,000	75.3	82.8	68.6	14.2
1,500	73.6	80.6	67.4	13.2
2,000	72.0	78.6	66.1	12.5
2,500	70.3	76.7	64.7	12.0
3,000	68.7	74.9	63.3	11.6
3,500	67.1	73.2	61.7	11.5
4,000	65.5	71.6	60.1	11.5
4,500	64.0	70.1	58.5	11.6
5,000	62.4	68.8	56.8	12.0
5,500	61.0	67.5	55.0	12.5
6,000	59.5	66.3	53.1	13.2
6,500	58.0	65.2	51.2	14.0
7,000	56.5	64.3	49.3	15.0
7,500	55.1	63.6	47.3	16.3

TABLE 5. Showing Estimated Population of Parishes, Total and Malarial Death-rates, etc.

KINGSTON (including Port Royal)							ST. ANDREW					ST. THOMAS						
	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths
1898 ...	51,330	1,491	29.0	136	2.6	9.1	41,052	1,126	27.4	172	4.1	15.2	35,775	944	26.3	224	6.2	23.7
1899 ...	51,693	1,414	27.3	104	2.01	7.3	41,447	1,058	25.5	138	3.3	13.0	36,338	854	23.5	233	6.6	27.2
1900 ...	52,274	1,453	27.7	128	2.4	8.8	41,838	1,164	27.8	210	5.0	18.0	36,928	1,007	27.5	297	8.0	29.4
1901 ...	52,475	1,421	27.0	115	2.1	8.0	42,161	1,074	25.4	156	3.7	14.5	37,299	901	24.1	250	6.7	27.7
1902 ...	53,174	1,455	27.3	183	3.4	12.5	42,665	1,096	25.6	210	4.9	19.1	38,044	905	23.7	257	6.7	28.3
1903 ...	53,750	1,365	25.3	96	1.7	7.0	43,386	880	20.2	130	2.9	14.7	38,692	785	20.2	212	5.4	27.0
1904 ...	54,258	1,607	29.6	109	2.0	6.7	43,739	1,343	30.7	198	4.5	14.7	39,046	1,146	29.3	325	8.3	28.3
1905 ...	54,668	1,611	29.5	127	2.3	7.8	43,926	1,351	30.7	162	3.6	11.9	39,397	955	24.2	197	5.0	20.6
1906 ...	55,068	1,544	28.0	177	3.2	11.4	44,256	1,287	29.0	191	4.3	14.8	40,028	995	24.8	237	5.9	23.8
1907 ...	54,880	2,040	37.1	161	2.9	7.8	44,256	1,660	37.5	183	4.1	11.0	40,298	1,077	26.7	280	6.9	25.9
Mean	—	—	28.7	—	2.4	8.6	—	—	27.9	—	4.0	14.6	—	—	25.0	—	6.5	26.1

PORTLAND							ST. MARY					ST. ANN						
	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths
1898 ...	35,858	856	23.8	136	3.7	15.8	48,653	990	20.3	183	3.7	18.4	62,728	1,161	18.5	235	3.7	20.2
1899 ...	36,546	798	21.8	149	4.0	18.6	49,601	1,030	20.7	209	4.0	20.2	64,296	1,024	15.9	166	2.5	16.2
1900 ...	37,231	945	25.3	245	6.5	25.9	50,816	1,054	20.7	285	5.6	27.0	65,849	1,328	20.1	279	4.2	21.0
1901 ...	37,817	910	24.0	215	5.6	23.6	51,671	1,185	22.9	298	5.7	25.1	67,133	1,225	18.2	276	4.1	22.4
1902 ...	38,727	852	22.0	184	4.7	21.5	52,861	1,203	22.7	281	5.3	23.3	68,775	1,139	16.5	196	2.8	17.2
1903 ...	39,586	903	22.6	193	4.8	21.3	54,093	1,228	22.7	319	5.8	23.5	70,415	1,210	17.0	289	4.1	23.8
1904 ...	39,991	1,284	32.1	299	7.4	23.2	54,621	1,732	31.7	480	8.7	27.7	71,642	1,528	21.3	297	4.1	19.4
1905 ...	40,431	1,040	25.7	239	5.9	22.9	55,201	1,490	26.9	419	7.5	28.1	72,879	1,342	18.4	227	3.1	16.9
1906 ...	41,433	942	22.7	244	5.8	25.9	56,450	1,415	25.0	404	7.1	28.5	74,655	1,140	15.2	207	2.7	18.1
1907 ...	41,979	1,099	26.1	225	5.3	20.4	57,462	1,457	25.3	365	6.3	24.9	76,240	1,411	18.5	271	3.5	19.2
Mean	—	—	24.6	—	5.3	21.9	—	—	23.8	—	5.9	24.6	—	—	17.9	—	3.4	19.4



TABLE 5—continued.

TRELAWNY							ST. JAMES					HANOVER				
	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths
1898 ...	33,786	853	25.2	149	4.4	17.4	37,661	821	21.7	209	5.5	836	23.4	192	5.3	22.9
1899 ...	34,249	819	23.9	133	3.8	16.2	38,137	793	20.7	188	4.9	820	22.6	172	4.7	20.9
1900 ...	34,793	917	26.3	118	3.3	12.8	38,599	905	23.4	183	4.7	811	21.9	132	3.5	16.2
1901 ...	35,095	880	25.0	129	3.6	14.6	38,967	827	21.2	168	4.3	924	24.7	204	5.4	22.0
1902 ...	35,653	885	24.8	153	4.2	17.2	39,525	809	20.4	213	5.3	915	24.0	230	6.0	25.1
1903 ...	36,206	778	21.4	116	3.2	14.9	39,992	795	19.8	173	4.3	872	22.5	203	5.2	20.6
1904 ...	36,672	979	26.6	117	3.2	11.9	40,400	928	22.9	202	5.0	971	24.7	180	4.5	18.5
1905 ...	37,081	930	25.0	99	2.6	10.6	40,724	927	22.7	183	4.4	1,081	27.3	179	4.5	16.4
1906 ...	37,720	806	21.1	113	2.9	14.0	41,457	844	20.3	199	4.8	985	24.5	190	4.7	19.2
1907 ...	38,265	908	23.2	131	3.3	14.4	41,880	1,085	25.9	226	5.3	1,140	28.1	205	5.0	17.9
Mean	—	—	24.2	—	3.4	14.4	—	—	21.9	—	4.8	—	24.3	—	4.8	19.9

WESTMORELAND							ST. ELIZABETH					MANCHESTER				
	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Total deaths from all causes	Total death-rate	Total deaths from mal.	Death-rate mal.	Percentage of mal. deaths to total deaths
1898 ...	59,725	1,347	22.5	416	6.9	30.8	72,464	1,484	20.4	285	3.9	1,137	17.3	112	1.7	9.8
1899 ...	60,690	1,283	21.1	334	5.5	26.0	74,028	1,284	17.3	195	2.6	1,005	14.9	77	1.1	7.6
1900 ...	61,974	1,346	21.7	365	5.8	27.1	76,020	1,510	19.8	225	2.9	1,075	15.6	101	1.4	9.4
1901 ...	62,905	1,236	19.6	324	5.1	26.2	77,553	1,326	17.0	209	2.6	1,085	15.4	112	1.5	10.3
1902 ...	64,010	1,481	23.1	441	6.8	29.7	79,223	1,497	18.8	302	3.8	1,125	15.6	163	2.2	14.4
1903 ...	65,195	1,211	18.5	388	5.9	32.0	81,233	1,259	15.4	225	2.7	1,055	14.2	103	1.3	9.7
1904 ...	66,489	1,394	20.9	371	5.5	26.6	83,120	1,524	18.3	209	2.5	1,128	14.9	116	1.5	10.2
1905 ...	67,294	1,703	25.1	388	5.7	22.7	84,546	1,718	20.3	218	2.5	1,481	19.2	105	1.3	7.0
1906 ...	68,313	1,437	21.0	434	6.3	30.2	86,239	1,573	18.2	223	2.5	1,254	16.0	121	1.5	9.6
1907 ...	69,281	1,685	24.3	439	6.3	26.0	87,691	1,785	20.3	270	3.0	1,687	21.2	207	2.6	12.2
Mean	—	—	21.7	—	5.9	27.7	—	—	18.5	—	2.9	—	16.4	—	1.6	10.0

TABLE 5—continued.

	CLARENDON						ST. CATHERINE						WHOLE ISLAND					
	Estimated Pop.	Total deaths from all causes	Total death- rate	Total deaths from mal.	Death- rate mal.	Per- centage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death- rate	Total deaths from mal.	Death- rate mal.	Per- centage of mal. deaths to total deaths	Estimated Pop.	Total deaths from all causes	Total death- rate	Total deaths from mal.	Death- rate mal.	Per- centage of mal. deaths to total deaths
1898 ...	65,121	1,538	23.6	398	6.1	25.9	73,060	1,890	25.8	433	5.9	22.9	718,347	16,474	22.9	3,285	4.5	19.9
1899 ...	66,306	1,452	21.8	339	5.1	24.0	74,158	1,656	22.3	352	4.7	21.2	730,725	15,290	20.9	2,789	3.8	18.2
1900 ...	67,577	1,509	22.3	356	5.2	23.5	75,456	1,856	24.5	472	6.2	25.4	745,104	16,880	22.6	3,396	4.5	20.1
1901 ...	68,692	1,418	20.6	361	5.2	25.4	76,295	1,911	25.4	500	6.5	26.1	755,730	16,243	21.4	3,317	4.3	20.4
1902 ...	70,088	1,459	20.8	434	6.1	29.7	77,537	1,935	24.9	535	6.8	27.6	770,242	16,756	21.7	3,782	4.9	22.5
1903 ...	71,548	1,360	18.9	345	4.8	25.3	78,952	1,712	21.6	385	4.8	22.4	785,434	15,413	19.6	3,177	4.0	20.6
1904 ...	72,819	1,677	23.0	379	5.2	22.5	79,974	2,352	29.4	540	6.7	22.9	797,528	19,593	24.5	3,822	4.9	19.5
1905 ...	73,566	1,921	26.1	322	4.3	16.7	80,508	2,313	28.7	544	6.7	23.5	806,690	19,863	24.6	3,409	4.2	17.1
1906 ...	74,792	1,624	21.7	354	4.7	24.1	81,734	2,025	24.7	530	6.4	26.1	820,437	17,871	21.7	3,624	4.4	20.2
1907 ...	75,424	2,295	30.4	498	6.6	21.6	82,728	2,394	28.9	633	7.6	26.4	830,261	21,723	26.1	4,094	4.9	18.8
Mean	—	—	20.5	—	5.3	23.8	—	—	25.6	—	6.2	24.4	—	—	22.6	34,695	4.4	19.7

TABLE 6. Return showing the admissions from Malaria into the various Hospitals of the Island, from 1898 to 1908.

District	Hospital	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908
St. Thomas	Morant Bay	123	56 return	83	102	103	187	109	161	131	118	—
Portland	Hordley	No	997	764	670	399	649	175	241	377	201	—
"	Port Antonio	—	280	234	173	184	425	528	645	604	1,352	1,694
St. Mary	Buff Bay	No	detailed	return	204	242	435	357	225	157	419	396
"	Annotto Bay*	120	160	248	204	242	435	514	532	302	326	—
St. Ann	Port Maria	40	21	54	28	31	31	32	36	39	43	—
"	St. Ann's Bay	4	3	2	3	2	3	3	5	1	2	—
Trelawny	Cave Valley	47	21	87	83	29	26	100	71	20	25	—
St. James	Falmouth	66	31	55	69	41	31	29	33	26	27	—
Hanover	Montego Bay	—	10	13	14	23	66	23	33	33	10	27
Westmoreland	Lucea	—	186	173	119	31	28	34	32	345	359	420
St. Elizabeth	Savanna-la-Mar	52	45	44	37	21	27	35	37	38	53	36
Manchester	Black River	No	return	21	15	18	30	15	43	93	79	64
Clarendon	Mandeville	—	22	21	15	18	30	15	43	93	79	64
"	Chapelton	No	return	474	411	272	368	382	553	638	464	525
"	Lionel Town	—	289	733	1,205	557	571	625	840	1,066	702	122
"	Spanish Town	—	525	—	—	—	—	—	—	—	—	—
"	Kingston	—	—	—	—	—	—	—	—	—	—	—
	Totals.....	—	2,646	2,985	3,133	1,953	2,877	2,961	3,487	4,940	4,180	—

\* 12,470 cases of malaria recorded from March 1, 1898, to 30 November, 1908

TABLE 7. Showing number of admissions from malaria in all the public hospitals, Jamaica, 1907-08.

	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Total	Deaths	Per-nicious Malaria	Deaths
Morant Bay .....	6	4	1	9	11	15	8	13	24	25	17	19	152	3	2	2
Hordley.....	7	10	10	18	22	23	25	9	38	17	22	19	220	2	—	—
Port Antonio .....	32	26	35	106	218	249	243	163	206	202	86	45	1,611	7	—	—
Buff Bay .....	7	7	16	42	60	83	45	61	64	61	32	29	507	8	2	2
Annotto Bay .....	92	60	66	93	55	85	108	186	342	343	218	132	1,780	18	—	—
Port Maria .....	24	7	13	21	22	32	32	45	85	73	47	43	444	4	3	2
St. Ann's Bay .....	2	3	2	3	3	2	3	5	10	6	7	3	49	—	—	—
Cave Valley .....	—	—	—	—	—	—	—	—	—	1	1	—	2	—	1	—
Falmouth .....	—	3	—	2	2	6	2	6	1	—	—	1	23	5	2	1
Montego Bay .....	4	—	2	—	3	3	9	3	4	4	2	2	36	—	—	—
Lucea .....	5	4	2	2	6	3	2	2	2	3	—	—	31	3	—	—
Sav.-la-Mar .....	19	13	18	40	33	27	47	42	37	38	41	33	388	—	—	—
Black River .....	1	—	2	3	6	4	14	9	10	5	5	1	60	—	—	—
Mandeville .....	—	—	1	—	—	—	—	1	2	—	1	—	5	—	—	—
Chapelton .....	6	3	7	3	7	7	7	3	15	19	7	5	89	1	—	—
Lionel Town .....	138	89	50	81	59	31	60	47	84	122	85	87	913	3	—	—
Spanish Town .....	24	19	31	54	41	58	38	49	72	55	45	28	514	35	21	13
Totals .....	367	248	256	477	548	628	643	644	906	974	616	447	6,844	89	31	20
Kingston P.H. ....	45	55	48	71	87	64	66	67	53	52	33	25	666	32	—	1
Grand totals .....	412	303	304	548	635	692	709	711	1,049	1,026	649	472	7,510	121	31	21



TABLE 8. Table showing the amount of working days lost through illness on various estates.

Estate	District	1907				1908			
		Total No. of working days in period	Average daily No. of men employed	Total No. of working days spent in hospital	Percentage of working days lost through sickness	Total No. of working days in period	Average daily No. of men employed	Total No. of working days spent in hospital	Percentage of working days lost through sickness
A	St. Thomas ...	10,920	35	266	2.4	11,130	35	1,499	13.5
B	Portland .....	6,552	21	693	10.5	6,678	21	504	7.5
C	" .....	13,416	43	2,760	20.5	24,960	80	10,431	41.7
D	St. Mary .....	9,672	31	1,101	11.3	12,084	38	1,686	13.3
E	" .....	13,728	44	1,870	14.4	12,042	39	2,544	20.5
F	" .....	4,368	14	834	19.0	2,862	9	411	14.3
G	" .....	4,056	13	516	12.9	5,088	16	768	11.1
H	Westmoreland	6,240	20	1,498	24.0	7,950	25	2,170	27.2
I	" .....	6,240	20	848	13.5	6,042	19	958	15.8
K	Clarendon ...	26,208	70	4,999	19.0	18,126	57	1,544	8.4
L	" .....	24,648	79	4,103	16.6	24,464	77	3,014	12.3
M	" .....	13,728	44	2,233	16.2	13,356	42	1,031	7.7
		139,776	—	21,721	15.5	144,782	—	26,560	18.3

Percentage of days lost through sickness during two years, 16.9.

TABLE 9. Showing number of cases of malaria among the Jamaica constabulary

Parish and constabulary station	1907			1908		
	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria
<b>KINGSTON</b>						
Sutton Street .....	31	170	18.2	36	170	21.1
Port Royal .....	—	6	—	—	6	—
Rock Fort .....	—	3	—	—	3	—
Rae Town .....	—	4	—	—	4	—
Brown's Town .....	—	4	—	—	4	—
Franklin Town .....	—	4	—	—	4	—
South Camp Road .....	—	4	—	—	4	—
Allman Town .....	—	4	—	—	4	—
Fletcher's Town .....	—	4	—	—	4	—
Hannah's Town .....	—	4	—	—	4	—
Smith's Village .....	—	4	—	—	5	—
Water Police .....	1	15	—	1	15	—
<b>ST. ANDREW</b>						
Half Way Tree .....	19	32	59.3	16	41	39.0
Cross Roads .....	11	24	45.8	9	28	32.1
Mathilda's Corner .....	2	11	17.2	—	13	—
Gordon Town .....	3	7	42.8	1	7	14.2
Guava Ridge .....	—	5	—	1	5	—
Stoney Hill .....	2	9	—	—	9	—
Laurence Tavern .....	1	10	10.0	3	10	30.0
Bull Bay* .....	11	14	—	—	3	—
<b>ST. THOMAS</b>						
Morant Bay .....	22	14	157.1	31	13	238.4
Bath .....	2	3	—	2	3	—
Golden Grove .....	3	3	—	3	3	—
Port Morant .....	3	2	—	5	2	—
Yallas .....	3	3	—	3	3	—
Llandewey .....	1	2	—	1	2	—
Trinity Ville .....	1	3	—	0	3	—
Cedar Valley .....	—	3	—	—	3	—
Hagley Gap .....	1	2	—	1	2	—
<b>PORTLAND</b>						
Port Antonio .....	49	25	196.0	37	28	132.1
Manchioneal .....	5	4	—	—	4	—
Castle .....	4	3	—	2	3	—
Buff Bay .....	10	4	250.0	6	5	120.0
Hope Bay .....	4	4	—	2	4	—
St. Margaret's Bay .....	3	3	—	2	2	—
<b>ST. MARY</b>						
Port Maria .....	46	20	230.0	56	20	280.0
Annotto Bay .....	61	10	610.0	68	10	680.0
Castleton .....	1	3	—	—	2	—
Richmond .....	5	5	—	7	6	—
Lucky Hill .....	2	3	—	1	3	—
Retreat .....	—	3	—	2	3	—
Oracabessa .....	3	3	—	3	3	—

\* Section closed 1 February, 1908

TABLE 9—continued

Parish and constabulary station	1907			1908		
	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria
<b>ST. ANN</b>						
St. Ann's Bay .....	13	14	92.8	9	14	64.2
Brown's Town .....	—	8	—	—	8	—
Moneague .....	1	4	—	—	3	—
Claremont .....	—	3	—	3	3	—
Ocho Rios .....	1	3	—	4	3	—
Dry Harbour .....	1	2	—	1	2	—
Cave Valley .....	1	3	—	—	3	—
Alexandria .....	—	3	—	—	3	—
Runaway Bay .....	—	2	—	—	2	—
<b>TRELAWNY</b>						
Falmouth .....	11	13	86.6	6	14	42.8
Deeside .....	4	2	—	1	2	—
Duncans .....	4	3	—	—	3	—
Rio Buono .....	1	2	—	—	2	—
Clark's Town .....	2	4	—	3	4	—
Stewart Town .....	5	3	—	—	3	—
Ulster Spring .....	2	3	—	—	3	—
<b>ST. JAMES</b>						
Montego Bay .....	7	31	22.7	18	32	56.2
Montpelier .....	2	4	—	1	4	—
Adelphi .....	3	4	—	2	4	—
Spring Mount .....	2	3	—	1	3	—
<b>HANOVER</b>						
Lucea .....	3	15	20.0	5	14	35.7
Green Island .....	6	3	200.0	12	4	300.0
King's Vale .....	—	2	—	—	2	—
Sandy Bay .....	4	3	—	9	3	—
Miles Town .....	—	2	—	—	3	—
<b>WESTMORELAND</b>						
Savanna-la-Mar .....	37	18	206.2	21	18	117.2
Morgan's Bridge .....	4	4	—	2	4	—
Little London .....	3	3	—	4	3	—
Negril .....	—	1	—	—	1	—
Bluefields .....	1	3	—	1	3	—
Whithorn .....	1	5	—	—	5	—
Bethel Town .....	—	4	—	—	4	—

TABLE 9—continued

Parish and constabulary station	1907			1908		
	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria	No. of cases of malaria at station	No. of S.O's and men at station	Percentage of cases of malaria
<b>ST. ELIZABETH</b>						
Black River .....	44	28	157·1	24	30	80·0
Balaclava .....	—	6	—	—	7	—
Santa Cruz .....	3	7	—	2	6	—
Lacovia .....	9	9	—	—	8	—
Malvern .....	—	5	—	—	6	—
New Market.....	—	5	—	—	5	—
Siloah .....	1	5	—	2	4	—
Pedro Plains.....	—	6	—	—	5	—
<b>MANCHESTER</b>						
Mandeville .....	—	13	—	—	12	—
Christiana .....	—	4	—	—	4	—
Cottage .....	—	3	—	1	3	—
Kendal .....	—	3	—	—	3	—
Porus .....	3	3	—	—	3	—
New Port .....	2	3	—	2	3	—
Williamsfield .....	2	2	—	—	2	—
Asia .....	—	2	—	—	2	—
Alligator Pond .....	2	2	—	2	2	—
<b>CLARENDON</b>						
Chapelton .....	13	14	92·8	9	14	64·2
May Pen .....	18	11	163·6	31	11	272·7
Alley .....	15	5	300·0	17	5	340·0
Four Paths .....	—	3	—	2	3	—
Hayes .....	2	1	—	3	1	—
Milk River .....	1	1	—	—	1	—
Frankfield .....	—	3	—	1	3	—
Rock River .....	—	2	—	1	2	—
Crofts Hill .....	—	2	—	2	2	—
<b>ST. CATHERINE</b>						
Spanish Town .....	31	36	86·1	47	34	138·2
Linstead .....	12	16	75·0	16	15	106·6
Old Harbour .....	12	9	133·3	16	8	200·0
Old Harbour Bay .....	—	1	—	—	1	—
Riversdale .....	6	8	—	9	6	—
Ewarton .....	—	4	—	—	4	—
Point Hill .....	—	7	—	—	4	—
Guy's Hill .....	—	7	—	—	4	—
Shady Grove .....	—	5	—	—	3	—
Port Henderson .....	3	3	—	—	4	—
The Depot .....	141	226	62·3	232	267	86·8
Spanish Town .....	—	—	—	—	—	—
Total .....	749	—	—	820	—	—

1,569



TABLE 10. Showing Spleen Rate and Average Spleen in Various Parishes

District	Locality	Feet	Number of children Exam- ined	Spleens				Total with enlarged spleens	Spleen rate per cent.	Average spleen
				1	3	6	9			
St. Thomas ...	Albion .....	Sea level	10	2	1	6	1	8	80.0	5.0
	Yallas.....	"	34	25	9	—	—	9	26.4	1.5
			44	27	10	6	1	17	38.5	2.3
Portland .....	Port Antonio, East .....	Sea level	11	2	8	—	1	9	81.8	3.1
	" " " " .....	"	38	12	21	5	—	26	68.4	2.7
	Bound Brook, West ...	"	15	3	12	—	—	12	80.0	2.6
	Bound Brook School ...	"	53	22	30	1	—	31	58.4	2.2
	Titchfield School, Lower Division .....	"	99	46	45	8	—	53	53.5	2.3
	Titchfield School, Inter- mediate Division ...	"	50	22	25	3	—	28	56.0	2.3
	Titchfield School, Upper Division .....	"	6	4	2	—	—	2	33.3	1.6
	Windsor .....	200	10	0	8	2	—	10	100.0	4.6
	Stanton .....	"	6	1	1	4	—	5	83.3	4.6
	Louis Hope .....	"	3	—	—	3	—	3	100.0	8.0
			291	112	152	26	1	179	61.5	2.5
St. Mary .....	Annotto Bay .....	Sea level	106	32	60	12	2	74	69.8	2.85
	Epsom School .....	100	34	23	11	—	—	11	32.3	1.6
	Enfield .....	200	29	23	6	—	—	6	26.0	1.4
	Fort Stewart .....	150	27	11	13	3	—	16	59.2	2.5
	Cape Clear .....	250	15	6	9	—	—	9	60.0	2.2
	Chovey .....	100	24	16	8	—	—	8	33.3	2.0
	Orange Hill .....	100	64	41	18	5	—	23	35.9	1.95
	Bremmerhall .....	slightly above	25	17	8	—	—	8	32.0	1.6
	Fontabelle .....	"	12	8	4	—	—	4	33.3	1.6
	Trinity .....	"	14	6	7	1	—	8	57.1	2.3
	Port Maria .....	Sea level	48	33	14	1	—	15	31.2	1.5
			398	216	158	22	2	182	45.7	2.1
St. Ann .....	Brown's Town.....	1000	69	69	—	—	—	0	Nil	1.0
Trelawny .....	Falmouth .....	Sea level	102	100	2	—	—	2	1.96	1.09
	Duan Vale .....	300 to 400	87	87	—	—	—	0	0	1.00
			189	187	2	—	—	2	1.06	1.03

TABLE 10—continued

District	Locality	Feet	Number of children Exam- ined	Spleens				Total with enlarged spleens	Spleen rate per cent.	Average spleen
				1	3	6	9			
Westmoreland	Savanna-la-Mar .....	Sea level	142	139	3	—	—	3	2.1	1.04
	Fontabelle .....	"	20	5	12	3	—	15	75.0	2.95
	Paradise.....	"	19	19	0	0	0	0	Nil	1.00
	Mounteagle .....	slightly above	19	14	2	2	1	5	26.2	2.15
	Bethel Town .....	1,000	78	78	0	0	0	0	Nil	1.00
			278	255	17	5	1	23	8.2	1.2
St. Elizabeth	Black River .....	Sea level	137	114	20	3	0	23	16.7	1.4
	Great Pedro Bay .....	"	51	22	25	4	0	29	54.9	2.3
	Newell .....	220	34	33	1	0	0	1	2.9	1.1
	" 1 mile off .....	200	27	26	1	0	0	1	3.7	1.2
			249	195	47	7	—	54	21.2	1.5
Manchester ...	Mandeville .....	2,200	42	42	0	0	0	0	Nil	1.0
Clarendon ...	Chapelton .....	500	44	44	0	0	0	0	Nil	1.0
St. Catherine	Linstead .....	200	28	23	4	1	0	5	17.8	1.46
	Braetown .....	Sea level	7	5	2	0	0	2	28.5	1.5
	Salt Pond .....	"	39	14	18	6	1	25	69.2	2.9
	Spanish Town .....	slightly above	138	114	24	0	0	24	17.3	1.3
			212	156	48	7	1	56	26.4	1.6
Kingston .....	Kingston .....	Sea level	220	197	23	0	0	23	10.4	1.2

(The levels are in most cases only approximate.)



View of the Rio Grande, showing flattening of river bed and shallow pool formation.



Anopheline breeding-swamp outside Black River on the road to Great Pedro Bay.



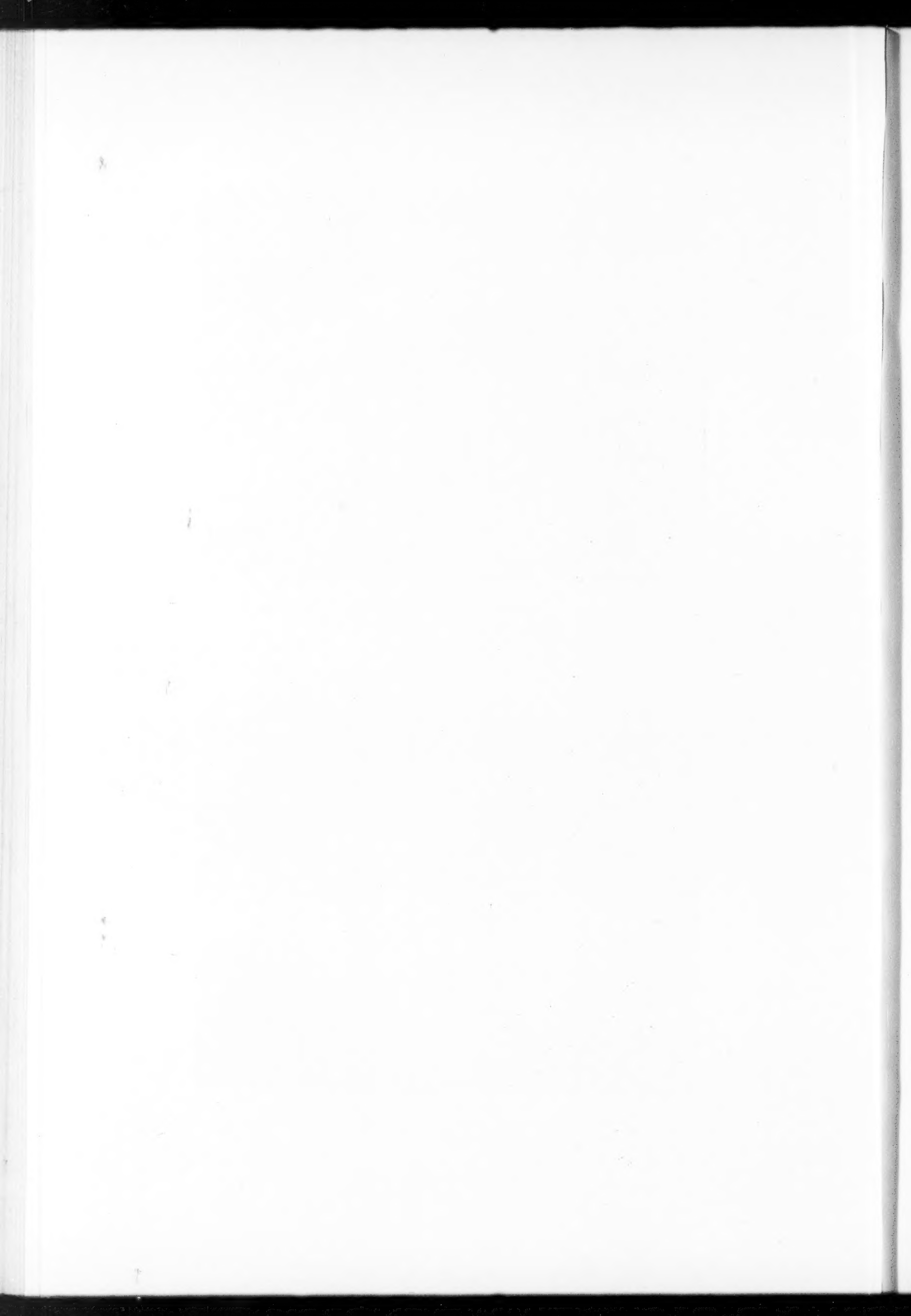


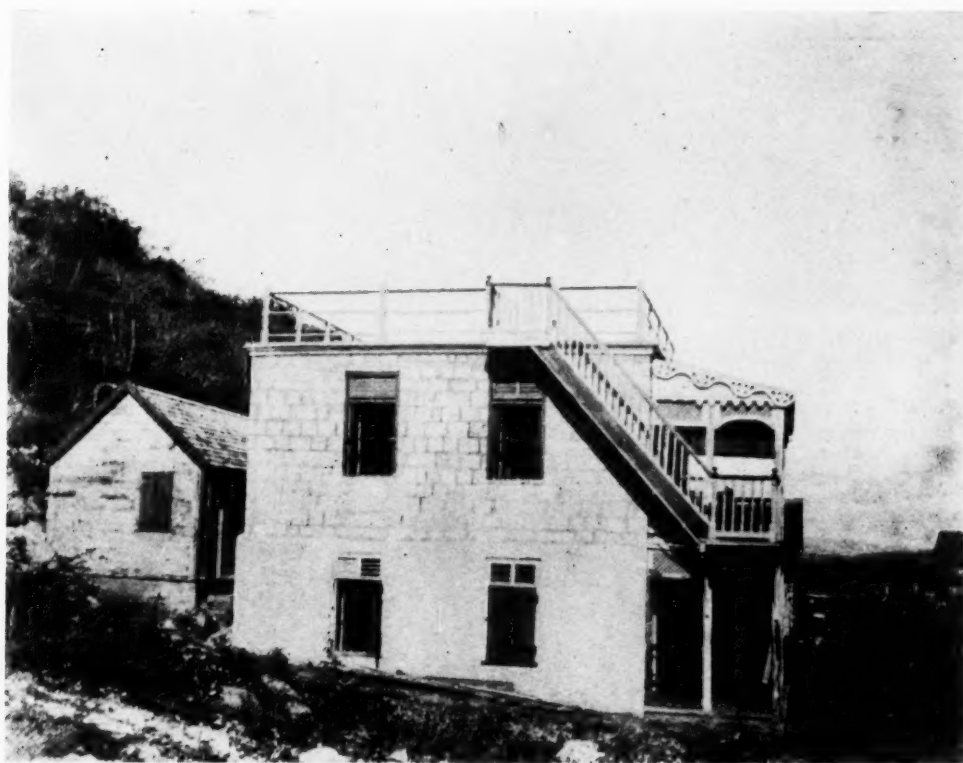


Edge of banana plantation at Port Antonio, showing Anopheline breeding-pool in the foreground.



Extensive Anopheline breeding-pool situated in the centre of the town of Annotto Bay.





The Police Station at Port Henderson. The only mosquito-proof house in the Island.





OBSERVATIONS ON THE LIFE HISTORY  
OF *TRYPANOSOMA LEWISI* IN THE  
RAT LOUSE (*HAEMATOPINUS*  
*SPINULOSUS*)

BY

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AND

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*(Received for publication 26 October, 1909)*

For over a year we have carried out experiments concerning the mode of transmission of *Trypanosoma lewisi* by means of the rat louse *Haematopinus spinulosus*. Although our results are far from complete, circumstances compel us to abandon this research for the present.

We have succeeded in transmitting *T. lewisi* by means of *Haematopinus spinulosus*, and, with the aid of wet fixation methods, in modifying conclusions which have previously been drawn concerning the cytological characters of this parasite in specimens stained by Romanowsky. We do not intend in the present paper to go into a detailed, theoretical discussion, which we reserve for a future communication, but only to bring forward some new facts and observations concerning the life-history of *T. lewisi* in its intermediate host.

Prowazek<sup>1</sup> was the first to draw attention to the life-history of *T. lewisi* in the rat louse. For some years Prowazek's conclusions were generally accepted, without being confirmed by other workers. Patton's and Strickland's<sup>2</sup> attempt to confirm Prowazek's work led them to the conclusion, that Prowazek had described part of the life cycle of a natural flagellate, *Crithidia* sp., of *Haematopinus spinulosus*, which they believe has no connection with *T. lewisi*.

Nuttall,<sup>3</sup> using thirty lice, was able to transmit, in the first of two experiments, *T. lewisi* from an infected to a normal animal. In the second experiment, ten lice were transferred from a wild to an uninfected rat without, however, giving rise to an infection in the latter. As he was unable to trace any development of *T. lewisi* in the rat louse, he seriously doubts whether any such development occurs.

Baldry,<sup>4</sup> on the other hand, working in Hartmann's laboratory was not only able to obtain positive results in his transmission experiments, but also fully confirms Prowazek's observations regarding conjugation. He asserts that the developmental cycle takes place within eight to ten days, and that it is a very small *Crithidia*-like form of *T. lewisi* in the louse which gives rise to the new infection.

#### TRANSMISSION EXPERIMENTS

In three of our experiments we have been able to transmit *T. lewisi* by means of the rat louse. We usually collected well-fed lice from a heavily infected rat and transferred them, after an interval of twenty hours, to a normal rat. In one experiment, twenty-four, and in the other two experiments, forty, lice were used. In the first two cases parasites appeared in the peripheral blood after an incubation period of eight days, and in the third after ten days. In each case the animal developed a typical infection.

In two further experiments ten lice were used after having been kept for two days in test tubes, but in neither did an infection result, although the animals were kept under observation for a considerable period.

*Method.* For the study of the parasite in the intermediate host, rat-lice were collected from an infected animal. The heads of the lice were snipped off with a small pair of scissors, and the gut contents teased out in a small amount of physiological saline solution. Usually a film was made of the gut contents of five to ten lice, and while still wet dipped into Flemming's or Hermann's solution, or sublimate-acetic-alcohol. Flemming's solution, however, gave the best and most satisfactory results, and parasites fixed in this way showed less deformity than by any other method. If sublimate-acetic-alcohol was used, the parasites always seemed to be somewhat shrunken.

The specimens were stained with Breinl's safranine-methylene-blue tannin orange method and Breinl's modification of Heidenhain,

as described in a previous paper.<sup>5</sup> The comparative use of these two methods was of great service. In the former method, granules, which were often present in large numbers, stained brilliantly with safranine, at times masking the nucleus and blepharoplast and often rendering the interpretation of cytological details difficult. When, on the other hand, Heidenhain's haematoxylin method was used the granules remained unstained. In this method, however, it was more difficult to obtain as perfect a differentiation of the details of the nucleus as with safranine methylene blue. Fresh examination was also constantly practised, and sometimes a parasite was kept under observation for as long as twenty-four hours.

It seems surprising that Nuttall and his co-workers were unable to observe developmental changes in rat-lice taken from rats infected with *T. lewisi*. However, for nearly four months we saw nothing but degenerated trypanosomes in our rat-lice, so that we nearly abandoned further research. Ultimately, in one case developmental forms were found in lice taken from an infected animal, and it became apparent that during the first stages of infection, so long as dividing and segmenting forms were present in the blood, the trypanosomes taken up by the louse only degenerated. On the other hand, so soon as division forms no longer appeared in the peripheral blood, the trypanosomes taken up by the louse underwent marked changes in those cases in which the rat's blood was literally swarming with trypanosomes. If, however, there were only comparatively few trypanosomes in the blood (about seventy to eighty to a microscopic field), hardly any changes were noticeable in the gut contents of the louse.

Thus, although nothing but degenerated trypanosomes were present in the gut contents of lice taken from rats in the early stages of infection, nevertheless, developmental forms made their appearance in the later stages of the disease, but only when the blood of the rat was literally swarming with trypanosomes.

The close resemblance of the parasites in the gut of the louse to typical *Herpetomonas* forms described by Prowazek, Patton, etc., naturally suggested that we were perhaps dealing with a mixed infection in the rat louse—a natural infection with *Herpetomonas*, and a secondary infection with *T. lewisi*.

We made films, however, of the gut contents of about 600 lice



taken from the same batch of normal rats which were afterwards used for infection, but in no instance have we been able to observe any parasites in the gut contents resembling the developmental forms of trypanosomes; only in rare instances were very small, round cells observed, which resembled gregarines. So far we have not been able to come to any definite conclusion concerning the nature of these bodies.

When lice in small numbers were placed on a clean rat, they were often rapidly eaten off by the animal and disappeared completely. On the other hand, the lice sometimes multiplied with great rapidity, so that the fur of the animal was soon swarming with them, and the animal itself soon succumbed to their injurious effects. Prowazek has already drawn attention to this difficulty.

*Fresh examination.*

For fresh examination, the gut contents of lice were teased out in physiological saline solution, and a coverslip preparation ringed with hard paraffin was made. In these specimens the trypanosomes could be kept alive in an actively motile condition for about thirty hours. It is a striking fact that if the lice were obtained from rats in an early stage of infection, the trypanosomes in the gut were of large size and moved sluggishly. When the rats were in a later stage of infection, the trypanosomes were small and moved at a very rapid pace, shooting across the microscopic field, so that detailed observation was almost impossible.

To obviate this difficulty a slide was first covered with a thin film of gelatine in saline solution, by which means the progressive movement of the trypanosomes was retarded. The gut contents of from three to four lice taken from a rat at a suitable stage of the infection were then taken, and specimens were made in the above described manner. The gut contents were seen to be full of parasites, some, large and moving sluggishly, others, small and resembling in their appearance living spermatozoa, possessing a small round head and a long wavy flagellum. In some instances a marked transformation was seen to take place. A large trypanosome became altered in shape; a constriction appeared at a point distant from one end about one-third of the length of the parasite. This gradually became more and more pronounced, at times disappearing, but, as a rule, in from two to four



hours becoming permanent. Then the movements of the parasites quickened, and a separation into two halves, which was only connected by a thin protoplasmic strand, occurred. The parasites often remained at this stage, no further changes occurring. In only a few instances was a further development seen to take place; the anterior part of the parasite appearing to disintegrate, until, finally, only a round head with a long rapidly moving flagellum remained. In only one case was the flagellum actually seen to become detached.

No further development occurred. We have never been able to observe any process which could be satisfactorily explained as representing conjugation. Now and again, parasites apparently consisting of two changed trypanosomes, joined at one end, were observed. Although these parasites were sometimes kept under observation for twenty-four hours until all movement had ceased, in no instance did any transformation occur which could be regarded as conjugation. Tempting as it is to regard certain appearances in stained specimens as conjugation forms, nothing but observation of the whole process in fresh specimens can be regarded as conclusive.

Small, round forms, as described later in stained specimens, have frequently been seen exhibiting a circular movement.

#### STAINED PREPARATIONS

Changes analogous to those which we have just described can be seen in stained specimens. For the preparation of these, lice were sometimes used which had been taken from an infected rat and kept alive in a test tube for as long as seventy-two hours. After this time they usually died, unless put back on another animal.

The trypanosomes in the gut of a louse taken shortly after the height of the infection was reached usually showed quite characteristic changes in the nucleus. Whereas in a normal blood trypanosome the nucleus only contained one intranuclear centrosome (karyosome), in the majority of the louse parasites, this had divided, and the division products had moved to opposite ends of the nucleus (Figs. 1-3).

At this stage forms were found in which the nucleus had undergone complete equal division, each half containing an intranuclear centrosome surrounded by a light unstained area of karyolymph.

In a further stage (Fig. 4), one of the halves of the nucleus had moved close to the blepharoplast (extranuclear centrosome), whereas the second half seemed to lose its staining properties, taking the haematoxylin stain in a somewhat diffuse manner. The parasites were markedly increased in size. At this stage the cytoplasm appeared to become denser in that end of the parasite in which the blepharoplast was situated (Fig. 5), whereas the cytoplasm of the opposite end exhibited a somewhat ragged appearance, and seemed to have lost its well-defined outline. At a still later stage, the meshes of the 'Schaumplasma' became larger and larger and less defined, and the cytoplasm about the flagellum more and more disintegrated (Fig. 6), until, finally, only the blepharoplast end was left with a long wavy flagellum. The half of the nucleus in the flagellar end lost its staining properties to a gradually increasing extent, and sometimes the cytoplasm contained a round, sharply-defined, darkly-staining vacuole, which was probably the remains of the degenerated half of the nucleus. The other half of the nucleus underwent further changes; it increased in size and changed its position with regard to the blepharoplast, in many instances appearing behind the blepharoplast. As a rule, it contained only one intranuclear centrosome, occasionally, however, two (Fig. 7).

Judging from the number of trypanosomes in the different stages of development, it is probable that after the division of the intranuclear centrosome (karyosome), the complete division of the nucleus takes place in a comparatively short time. Forms like Figs. 1-3 and Figs. 6-8 were often seen, whereas those shown in Figs. 4-5 were comparatively rare.

Slight modifications of this process were sometimes noticed. At times the parasite did not grow to the large size seen in Figs. 4-6, whilst at other times the divided nuclei remained connected by a thin strand for a considerable time (Fig. 10).

During these changes in the nucleus and cytoplasm of the parasite, the blepharoplast did not undergo any marked transformation beyond slight enlargement. In some cases, as in Figs. 5-8, it seemed to consist of a number of round granules, but not even in the best differentiated specimens could any detailed structure be made out.

At a later stage, parasites were seen resembling spermatozoa, consisting of an oval head with a long flagellum, as well as a few

forms without a flagellum. In all these stages a blepharoplast was present and could be clearly seen.

In other forms a new flagellum connected with the blepharoplast was observed. Whereas the original flagellum was thin and wavy, the new flagellum appeared very much thicker and bristle-like (Figs. 11, 12), but it seemed to become thinner as its length increased (Fig. 13). Up to this point, forms observed in stained specimens had always been controlled by examination of living parasites. Judging from the different size of the oval-shaped *Herpetomonas*-like forms of the developmental stages of *T. lewisi* (Figs. 11 and 15), one can assume that these forms increase in size.

If lice, containing a small number of these *Herpetomonas*-like forms, were taken from the rat and kept alive for a varying interval (we were not able to keep lice alive for longer than seventy-two hours), a marked multiplication of the forms took place in the gut (Fig. 18). Many of the latter, possessing only one nucleus and one blepharoplast, showed two flagella. We are inclined to regard these bi-flagellated forms as the first stage in the process of division.

The division appears to start with a broadening or rounding up of the blepharoplast (Figs. 14, 15). Afterwards, the nucleus divides and the cytoplasm of the parasite splits longitudinally into two daughter cells (Fig. 16).

Sometimes, however, before one division has come to an end, a second division has already commenced (Fig. 17). When these forms occur in large numbers, they can often be seen to be agglomerated in great clusters, with the flagellum always directed inwardly (Fig. 18). Very great variation in the shape and structure of the *Herpetomonas*-like forms was noticed (Figs. 19, 20).

In specimens stained with safranine-methylene blue, bright granules in varying numbers can very often be seen (Figs. 21, 22). Now and again the nucleus of these forms degenerates (Figs. 23, 24, 25). In Fig. 26 there is only a light area with irregular red granules in the position of the nucleus.

The stages depicted in Figs. 27 and 28 are suggestive of conjugation forms. Although analogous appearances were seen in fresh specimens and kept under observation for several hours, no further changes occurred.



*Round forms.* Frequently, especially in lice taken from rats at a later stage of infection, round forms, as in Figs. 32-36, were noted. They usually showed a typical oblong nucleus and a small blepharoplast, from which a long, round flagellum, which was usually wound round the parasite, originated. Whether these forms develop in the way suggested by Fig. 31, or whether they result from the coiling up of a normal trypanosome could not be determined.

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## DESCRIPTION OF PLATES

The figures have been drawn with an Abbé drawing apparatus, Apochromatic, 2 mm., ap: 1.4, and compensating ocular 18.

Figures 1, 2, 18, 21, 22, 26, 27 and 30 were stained with safranine-methylene-blue orange-tannin. All the others were stained by Heidenhain's method.

## PLATE XIX

Figs. 1-3. Early stage, showing division of intranuclear centrosome (karyosome).

Fig. 4. Complete nuclear division. One half shows signs of degeneration.

Figs. 5, 9.—Stages showing the disintegration of part of the cytoplasm.

Fig. 10.—Later stage of division of the nuclei, both halves of which are still connected.

Figs. 11, 12.—Stages showing the growth of a new flagellum.

Figs. 13-15.—*Herpetomonas*-like forms.

Figs. 16, 17.—Division of *Herpetomonas*-like forms.



A.M. 13. ad nat. del.

Hath, London.

## PLATE XX

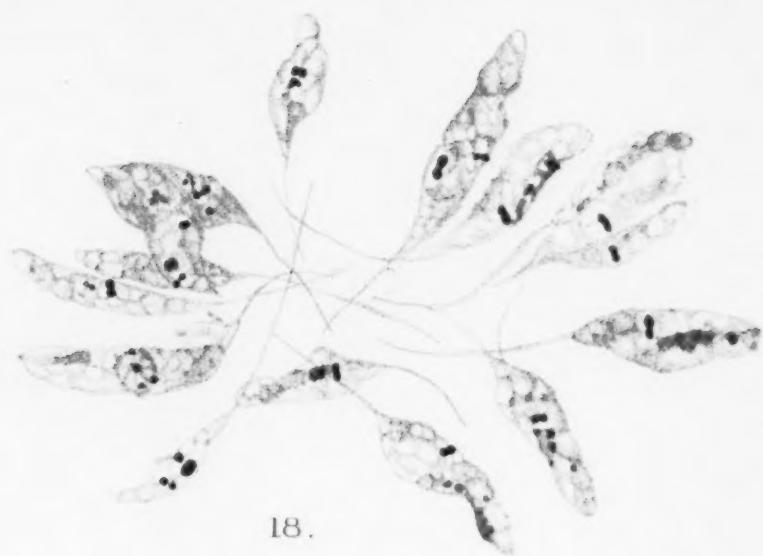
Fig. 18.—Conglomerate of *Herpetomonas*-like forms.

Figs. 19-30.—*Herpetomonas*-like forms.

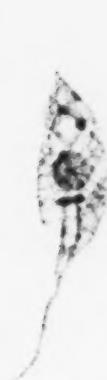
Fig. 31.—Formation of round form.

Figs. 32-36.—Round forms.

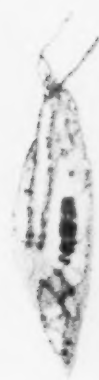




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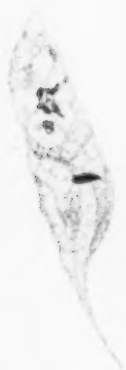
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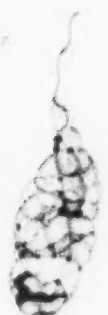
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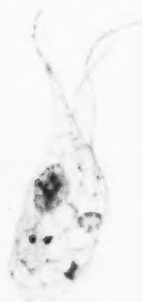
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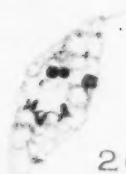
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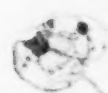
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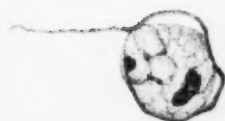
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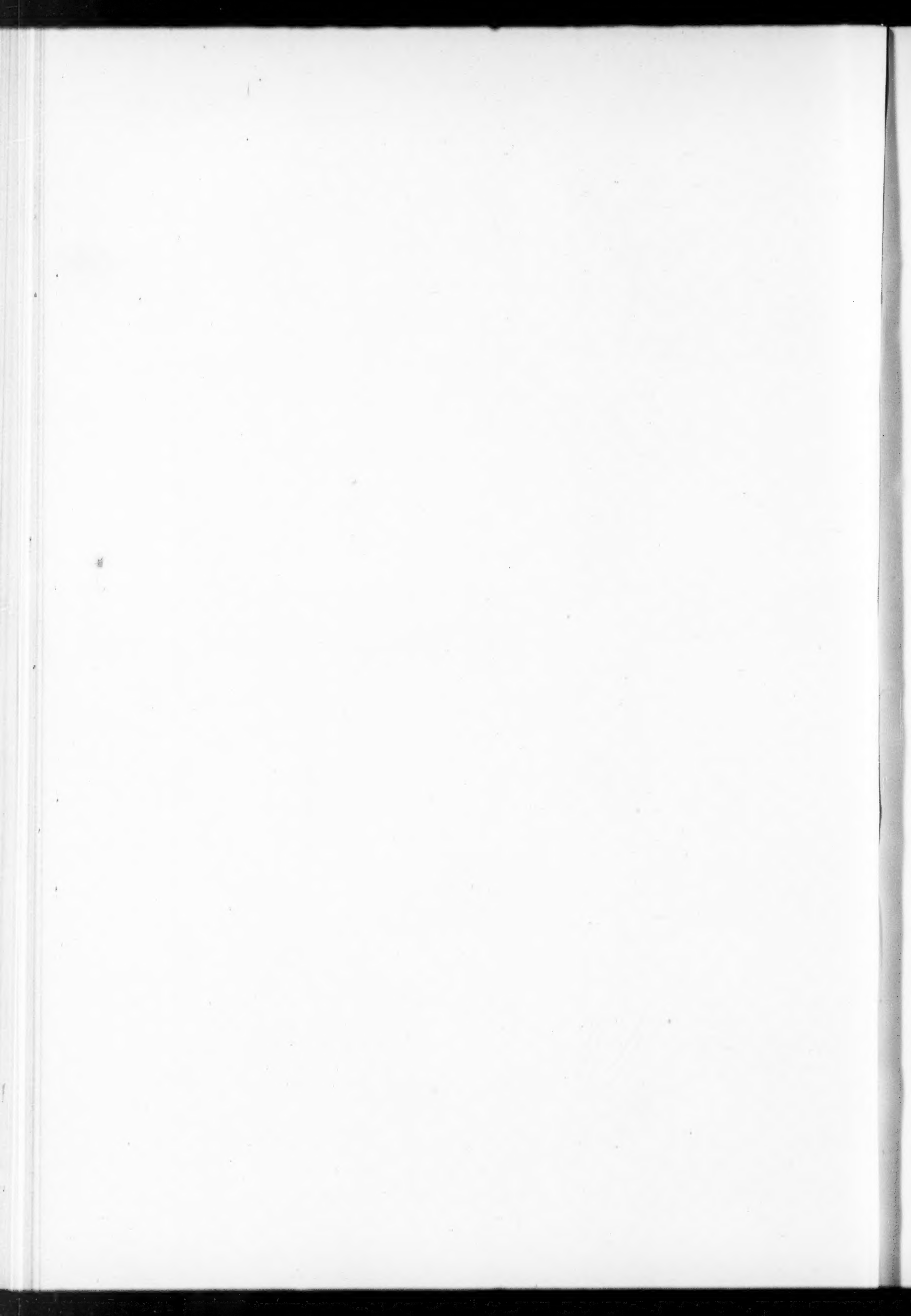
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AMB. ad nat. del.

Rich. London.



# ON THE VARIATION OF THE HAEMOLYTIC COMPLEMENT IN EXPERIMENTAL TRYPANOSOMIASIS

BY

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*(Received for publication 5 February, 1910)*

Hartoch and Yakimoff\* observed a diminution in the amount of haemolytic complement in the blood of guinea-pigs and rats infected with various strains of trypanosomes shortly before the death of the animals.

With a view to ascertaining whether this diminution of the haemolytic complement bore any definite relation to the number of trypanosomes present in the peripheral blood of the infected animal, or whether it was simply a terminal event of the disease, the following series of experiments was undertaken.†

For the purpose of preparing a haemolytic system about 50 c.c. of goat's red corpuscles were injected into the peritoneal cavity of a rabbit, all traces of serum having been previously removed from the red cells by washing three or four times with 0.9 per cent. NaCl solution. These injections were repeated once or twice at intervals of seven days. Usually at the end of two or three weeks the rabbit's serum was strongly haemolytic for goat's red cells. The immune rabbit-serum was then inactivated by heating to 56°-58° C. for half an hour.

In order to standardise the haemolytic system, amounts varying

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\* Wien klin. Woch., No. 40, 1908.

† The animal experiments recorded in this paper were kindly performed for me by Dr. Anton Breinl.

from 0.0005 c.c.-0.005 c.c. of the immune serum were added to 1 c.c. of a 5 per cent suspension of well-washed goat's red cells. Afterwards, 1 c.c. of a 1 in 10 dilution of normal guinea-pig serum was added, as complement, to each of these mixtures of amboceptor and red cells. The contents of each of the tubes was then made up to 3 c.c. by the addition of 0.9 per cent. NaCl solution. It was found that 0.001 c.c. of the immune serum was the smallest amount necessary to haemolyse 1 c.c. of a 5 per cent. suspension of goat's red cells in from 10-15 minutes at 37° C.

A haemolytic system consisting of the double amount of the immune serum 0.002 c.c. and 1 c.c. of a 5 per cent. suspension of goat's red cells was employed in the following experiments.

The serum of infected guinea-pigs was used throughout these experiments. The amount of complement present in the blood, withdrawn from one of the veins in the ear, was estimated before injection, and the guinea-pigs were then inoculated with different strains of trypanosomes, e.g., *T. gambiense*, *T. equiperdum*, *T. brucei* or *T. evansi*. Subsequently, examinations of the amount of haemolytic complement in their serum were made at intervals of two or three days during the course of the infection. An observation of the number of trypanosomes in the peripheral blood was made daily.

In Tables I and II the results obtained with the serum of guinea-pigs infected with *T. brucei* and *T. evansi*, respectively, are recorded.

Similar results were obtained with guinea-pigs 13 and 14 infected with *T. gambiense*, and with guinea-pigs 15 and 16 infected with *T. equiperdum*. The amount of haemolytic complement present in the serum of these animals was examined frequently during the period—between three and four and a half months—which they lived after infection. At no time was any diminution of the complement observed until a few hours before the animal's death, when a partial disappearance of complement was noticed in animals 13 and 15. There was no appreciable loss of complement observed in guinea-pigs 14 and 16 in the serum removed about twelve hours before their death. At no time did their blood exhibit more than one or two trypanosomes to a field, and frequently none were seen after a careful search.



TABLE I—Guinea-pigs infected with *T. brucei*.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
I	5 days before inoculation	Inoculated intraperitoneally with <i>T. brucei</i>		Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Trace of haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation			" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	2nd	5 parasites to a field	" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "	
	3rd								
	4th	10 "							
	5th	"							
	6th	"							
	7th	50 "							
	8th	Parasites numerous	" 30 "	Almost complete haemolysis in 3 hours	Very marked haemolysis in 3 hours	Trace of haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	
	9th	"	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "	" 3 "	
	10th	"	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	
	11th	40 to field							
	12th	Numerous*							
	13th	Found dead							

\* Animal very ill at the time the serum was removed.

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
2	2 days before inoculation			Complete haemolysis in 10 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Trace of haemolysis in 3 hours	No haemolysis in 3 hours
		Inoculated intraperitoneally with <i>T. brucei</i>		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	1st day after inoculation								
	2nd "	5 to a field		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	3rd "	2 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	4th "	5 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	5th "	10 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	6th "	15 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	7th "	5 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	8th "	1 to 5 fields		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	9th "	1 to 20 fields		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	10th "	1 to 5 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	11th "	1 to a field "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	12th "	5 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	13th "	10 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	14th "	15 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	15th "	40 "		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	16th "	Numerous		" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
	17th "		Complete haemolysis in 10 mins.						
	18th "	Innumerable*	Slight haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
	19th "								
	20th "	Found dead							

\* Animal very ill at the time the serum was removed.

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
3		Inoculated intra-peritoneally with <i>T. brucei</i>		Complete haemolysis in 10 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation								
	2nd "	5 to a field	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "
	3rd "								
	4th "		" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "
	5th "								
	6th "	30 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "
	7th "	20 "							
	8th "	1 to 5 fields							
	9th "								
	10th "	1 to 20 fields							
	11th "	1 to a field	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "
	12th "	5 "							
	13th "	10 "	" 10 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "	" 3 "
	14th "	15 "							
	15th "	40 "							
	16th "								
	17th "	Innumerable	Complete haemolysis in 20 mins.	" 60 "	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	" 3 "
	18th "		c.g.						
19th "		" "	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	" 3 "	" 3 "	" 3 "	

\* Animal dying when the serum was withdrawn.

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.					
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.005 c.c.
4								
		Inoculated intraperitoneally with <i>T. brucei</i>		Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours
	1st day after inoculation							No haemolysis in 3 hours
	2nd "							
	3rd "	1 to 2 fields	15 "	"	15 "	30 "	"	"
	4th "	10 to a field	"	"	"	"	3 "	3 "
	5th "	20 "	15 "	"	15 "	30 "	"	"
	6th "	30 "	"	"	"	"	3 "	3 "
	7th "	20 "	"	"	"	"	"	"
	8th "	20 "	15 "	"	15 "	30 "	"	3 "
	9th "							
	10th "	Found dead						

TABLE I—continued.



TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
5				Complete haemo- lysis in 15 mins.	Complete haemo- lysis in 15 mins.	Complete haemo- lysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemo- lysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation	Inoculated intra- peritoneally with <i>T. brucei</i>							
	2nd	4 to film							
	3rd	1 to field							
	4th	10 "	15 "	15 "	20 "	3 "	3 "	3 "	
	5th	40 "							
	6th								
	7th	Numerous							
	8th	"	15 "	15 "	30 "	3 "	3 "	3 "	
	9th	"							
	10th	"							
	11th	"	15 "	15 "	30 "	3 "	3 "	3 "	
	12th	"							
	13th	1 to a field							
	14th	2 "							
	15th	5 "							
	16th	10 "	15 "	15 "	30 "	3 "	3 "	3 "	
	17th	15 "							
	18th	"							
	19th	40 "	15 "	15 "	15 "	Almost complete haemolysis in 3 hours	Partial haemo- lysis in 3 hours	No haemolysis in 3 hours	" 3 "
	20th	Found dead							
21st									
6			Complete haemo- lysis in 10 mins.	Complete haemo- lysis in 15 mins.	Complete haemo- lysis in 15 mins.	Complete haemo- lysis in 30 mins.	Marked haemo- lysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation	Inoculated intra- peritoneally with <i>T. brucei</i>							
	2nd	1 to a field							
	3rd	10 "	15 "	15 "	30 "	3 "	3 "	3 "	
	4th	50 "							
	5th	50 "							
	6th	Numerous*							
	7th								

\* Animal dying when the serum was withdrawn.

TABLE I—Continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
7		Inoculated intraperitoneally with <i>T. brucei</i>		Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation								
	2nd								
	3rd								
	4th								
	5th	10 to a field	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	
	6th								
	7th	5 "							
	8th	1 to 5 fields							
	9th	1 to 10 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	
	10th	1 to 5 "							
	11th	1 to a field							
	12th	10 "							
	13th	10 "							
	14th	10 "							
	15th	10 "							
	16th	15 "							
	17th	40 "							
	18th	Numerous	Complete haemolysis in 10 mins.	" 15 "	" 15 "	" 20 "	" 3 "	No haemolysis in 3 hours	" 3 "
	19th	15 to a field							
	20th	50 "							
	21st	Numerous		" 20 "	" 20 "	" 30 "	" 3 "	Slight haemolysis in 3 hours	" 3 "
	22nd	" *	" 10 "	" 15 "	" 15 "	" 20 "	" 3 "	No haemolysis in 3 hours	" 3 "
	23rd	"	" 10 "	" 60 "	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	" 3 "

\* Animal dying when the serum was withdrawn.

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.										
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.				
8		Inoculated intraperitoneally with <i>T. brucei</i>											
	1st day after inoculation	1 to 20 fields											
	2nd "	1 to 10 "											
	3rd "	5 to field											
	4th "	2 "											
	5th "	15 "		15 "	15 "	30 "	3 "	3 "	3 "	3 "	3 "	3 "	No haemolysis in 3 hours
	6th "	5 "											
	7th "	1 "											
	8th "	5 "		15 "	15 "	30 "	3 "	3 "	3 "	3 "	3 "	3 "	
	9th "	15 "		15 "	15 "	30 "	3 "	3 "	3 "	3 "	3 "	3 "	
	10th "	Numerous		15 "	15 "	30 "	3 "	3 "	3 "	3 "	3 "	3 "	
	11th "		Complete haemolysis in 10 mins.										
	12th "	"	" 10 "										
	13th "	"											
	14th "		Found dead										

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
9				Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Trace of haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation	Inoculated intraperitoneally with <i>T. brucei</i>							
	2nd	1 to 40 fields							
	3rd	1 to 1/2 film							
	4th								
	5th	1 to 5 fields	15 "	"	15 "	30 "	3 "	"	3 "
	6th	1 to 15 fields							
	7th	1 to 30 fields							
	8th	1 to 1/2 film							
	9th	1 to 5 fields							
	10th	5-10 to field	15 "	"	15 "	30 "	3 "	"	3 "
	11th								
	12th	1 to 2 fields							
	13th	1 to 1/2 film							
	14th	1 to 1/2 film	15 "	"	15 "	30 "	3 "	"	3 "
	15th	1 to film							
	16th	1 to film							
	17th								
	18th								
	19th	Negative							
	20th	"							
	21st	"	15 "	"	15 "	30 "	3 "	"	3 "
	22nd	"							
	23rd								
	24th	1 to film							
	25th								
	26th	1 to 5 fields							
	27th	1 to 20 "							
	28th	1 to 5 "							
	29th	1 to 10 "							
	30th	1 to 5 "							
	31st	10 to field							
	32nd	15 "	15 "	"	15 "	30 "	3 "	"	3 "
	33rd	15 "							
	34th	50 "	15 "	"	15 "	30 "	3 "	"	3 "
	35th	Numerous							
	36th	"	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
	37th	"							

\* Animal moribund at the time the serum was withdrawn.



TABLE II.—Guinea-pigs infected with *T. evansi*.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
10		Inoculated intraperitoneally with <i>T. evansi</i>							
	1st day after inoculation	Negative							
	2nd "	1 to field							
	3rd "	5 "							
	4th "	10 "							
	5th "	Negative							
	6th "	2 to film							
	7th "	1 to 2 fields							
	8th "	20 to field							
11		Inoculated intraperitoneally with <i>T. evansi</i>							
	1st day after inoculation								
	2nd "	5 to field							
	3rd "	15 "							
	4th "	15 "							
	5th "	10 "							
	6th "	Found dead							
	7th "								

TABLE II—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
12			Complete haemolysis in 10 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
		Inoculated intraperitoneally with <i>T. evansi</i>							
	1st day after inoculation	Negative							
	2nd "	"							
	3rd "	2 to film	15 "	20 "	"	30 "	"	3 "	3 "
	4th "	"							
	5th "	1 to field							
	6th "	"							
	7th "	15 "	15 "	20 "	"	30 "	"	3 "	3 "
	8th "	15 "	"						
	9th "	15 "	"						
	10th "	20 "	"						
	11th "	30 "	15 "	20 "	"	30 "	"	3 "	3 "
	12th "	2 "	"						
	13th "	1 to 5 fields	"						
	14th "	1 to 10 fields	15 "	20 "	"	30 "	"	3 "	3 "
	15th "	5 to field	"						
	16th "	1 "	"						
	17th "	3 "	"						
	18th "	4 to film	"						
	19th "	5 "	"						
	20th "	Negative							
	21st "	"	15 "	20 "	"	30 "	"	3 "	3 "
	22nd "	"	"						
	23rd "	2 to film	"						
	24th "	2 "							
	25th "	Negative							
	26th "	1 to 5 fields	"						
	27th "	"							
	28th "	10 to field	10 "	20 "	"	30 "	"	3 "	3 "
	29th "	60 "	"						
	30th "	Numerous *	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours

\* Animal dying at the time the serum was withdrawn.

In none of the foregoing experiments was any decrease in the amount of haemolytic complement observed until shortly before the death of the animal, with the single exception of Experiment 1. The serum of this guinea-pig, which was infected with *T. brucei*, showed a slight decrease in complement on the 8th and 11th day of the disease. The blood at the time contained numerous trypanosomes.

In Experiments 2, 5, 7 and 8 no appreciable decrease of the haemolytic complement occurred during the course of the disease, even when the parasites were swarming in the peripheral blood (1 to every 2 or 3 red cells). Many estimations of the complement were made in the different experiments, when 20-50 parasites were present to the microscopic field (Zeiss objective DD; eyepiece No. 4). In no case was any diminution noticed. It is evident, therefore, that the presence of numerous trypanosomes in the peripheral blood in an early stage of the disease does not necessarily result in a diminution of the haemolytic complement.

On the other hand in a late stage of the infection shortly before the death of the animal, a considerable decrease in the amount of complement was observed in most of the experiments. On five occasions, Experiments 1, 2, 3, 9 and 12, the blood of animals infected with *T. brucei* and *T. evansi*, respectively, contained practically no haemolytic complement a few hours before death. In other cases, e.g., Experiments 6, 7, 13 and 15, only a partial diminution could be observed in the serum removed just before the death of the animal, whilst in still other cases, 5, 10, 11, 14 and 16, no decrease of the haemolytic complement was found in the serum withdrawn about twelve hours before death.

From these observations one must conclude, therefore, that the diminution of the complement in the serum of animals infected with trypanosomiasis is simply a terminal event, and does not occur during the earlier stages of the disease, even when the peripheral blood is swarming with parasites.

The following experiments were performed with a view to ascertaining whether the serum of infected guinea-pigs withdrawn a few hours before their death contained any body which had the property of inhibiting to any extent the activating power of the complement of normal serum.

EXPERIMENT.—The serum of guinea-pig 3 was obtained shortly before the death of the animal. It had not the slightest activating action even when added undiluted to the standardised haemolytic system of goat's red cells and amboceptor. A portion of this serum was added in varying proportions to the serum of a normal guinea-pig. The resulting mixtures were then kept at 37° C. for 20 minutes before being added to the haemolytic system. A second portion of the serum of guinea-pig 3 was first heated to 56°-58° C. for half an hour in order to destroy any complement it might contain, and then, as above, mixed in different proportions with normal guinea-pig serum.

The following Table gives the result of this experiment :—

A					
	5 % suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	0.9 % NaCl solution	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partial haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Trace of haemolysis in 3 hours

B					
	5 % suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	Serum guinea-pig 3	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partially complete haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Slight haemolysis in 3 hours

C					
	5 % suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	Serum of guinea-pig 3 (heated 56-58° C. for 20 mins.)	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partial haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Trace of haemolysis in 3 hours



Analagous results were obtained with the sera of guinea-pigs 9 and 12 withdrawn a few hours before their death. It is evident, therefore, that the addition of the sera of guinea-pigs 3, 9 and 12 (without complement) to normal guinea-pig serum caused no greater decrease in the activating power of the latter than occurred when the normal guinea-pig serum was diluted to a similar degree with 0.9 per cent. NaCl solution.

Possibly the diminution or absence of complement in the serum of animals dying from trypanosomiasis might be due to an absorption of the complement by the trypanosomes which at the time of death were usually present in the peripheral blood in very great numbers. The fact, however, that in the earlier stages of the disease, even when the trypanosomes were swarming in the peripheral circulation no diminution of haemolytic complement was noticed contravenes this explanation. Moreover, guinea-pigs 13 and 15, infected with *T. gambiense* and *T. equiperdum* respectively, exhibited a considerable decrease in haemolytic complement in the serum withdrawn a few hours before death, although the blood contained only one or two trypanosomes to the microscopic field. In this connection also, we have failed in our attempts to remove *in vitro* the haemolytic complement from normal guinea-pig serum by the addition of large numbers of well-washed trypanosomes obtained from heavily infected animals.

### CONCLUSIONS

The results obtained confirm those of Hartoch and Yakimoff, that in most cases of experimental trypanosomiasis, a marked diminution or total disappearance of the haemolytic complement can be observed for a few hours before the animal's death.

This decrease of the haemolytic complement is limited to the last stages of the disease, and is not met with in the earlier stages, even when the blood is swarming with trypanosomes.

The serum of an animal in the last stages of the disease, at a time when it contains no haemolytic complement, has no inhibitory effect upon the activating power of the complement of normal serum.

The presence of numerous trypanosomes in the blood causes of itself no diminution of the haemolytic complement, and secondly it is not possible to absorb, *in vitro*, the complement from normal serum by the addition of numerous trypanosomes.



## ACUTE CRAW-CRAW

BY

R. H. KENNAN, M.D., D.T.M.,

SENIOR MEDICAL OFFICER, WEST AFRICAN MEDICAL STAFF, SIERRA LEONE

*(Received for publication 7 February, 1910)*

The term 'Craw-Craw' is applied extensively in West Africa to describe pathological conditions of the skin, which are mainly characterised by the presence of a papulo-vesicular eruption, accompanied by more or less itching.

Generally, this rash is most marked about the buttocks, perineum, genitals, and groins; and is most commonly seen in a condition which seems to indicate chronicity, viz., scale formation, and hypertrophy.

But in certain cases I have seen the rash become almost universal with great rapidity, the outbreak being associated with fever and malaise, and under these conditions mistaken for variola and varicella.

Both at Accra, on the Gold Coast, and at Freetown, Sierra Leone, the affection in this form is not very uncommon.

The rash may be practically universal and is vesicular in the very early stage. The vesicles have very little, if any, papular foundation, and always lack the well-defined base of a typical variola spot, and, moreover, the spot is vesicular at the earliest period. Pricking the vesicle in the early stage allows exit of a sticky, pellucid fluid, and the vesicle can be entirely emptied by slight pressure without further rupture of its walls, leaving the latter collapsed. In the early stage of the case the great majority of the spots show an almost equal degree of development, but careful search will usually reveal some dried up, scaly spots. The centre of each of these old spots is generally of a lighter colour, and round the centre iris-like rings of epidermic scales, attached by their outer borders are seen. These represent a late stage of the

vesicular condition, and may, I believe, be the source from which auto-infection may become general.

A day or so after the first outbreak of vesicles, fresh vesicles are formed between the older ones, and fresh spots continue to appear for a week or so.

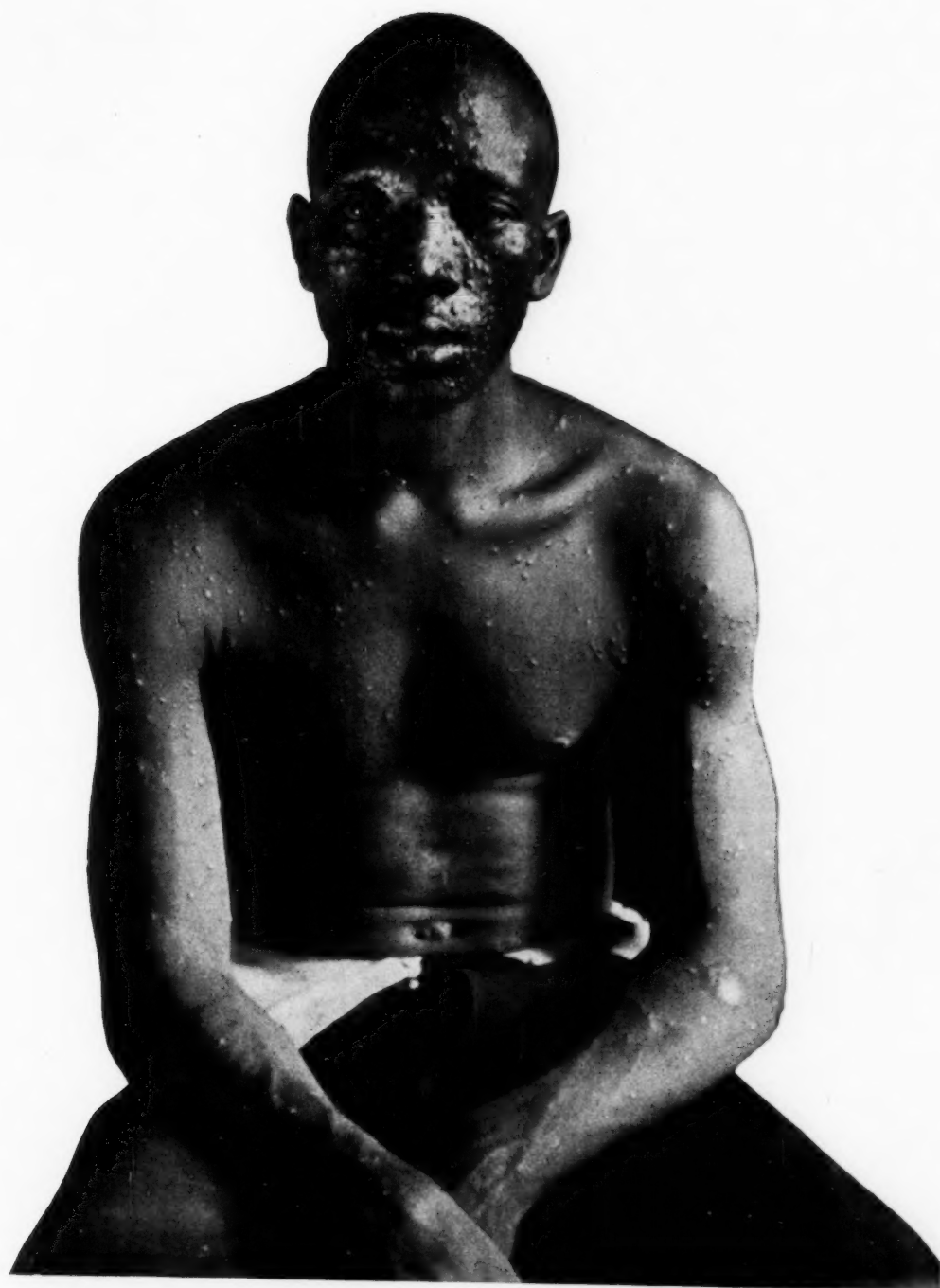
A few of the spots suppurate slightly, but definite adherent scabs are rare. A vesicle may show umbilication when a fine hair emerges from its centre.

The temperature is raised early, and before any suppuration has appeared, and there is no 'secondary' fever.

Constitutional symptoms are definite but slight, and no more than would be expected to accompany the moderate degree of fever usually present ( $100^{\circ}$  to  $102^{\circ}\text{F.}$ ), and which lasts for a few days only. Itching is not generally a marked symptom, and is usually not present till the later stage; it is rarely intense, and it usually ceases before the rash has entirely disappeared.

The face may be attacked but usually less so than other parts; the palms of the hands and soles of the feet usually escape; the fingers show few if any spots. Disappearance of the rash with desquamation of the epidermis of the affected parts is slow and protracted, and even with frequent bathing and friction the skin may still show signs of the rash for a month or six weeks, or even longer. No pitting or discoloration is found afterwards. The mucous membranes are not usually affected though I have seen spots on the prepuce and glans penis. The progress of vaccination is not influenced by the disease.



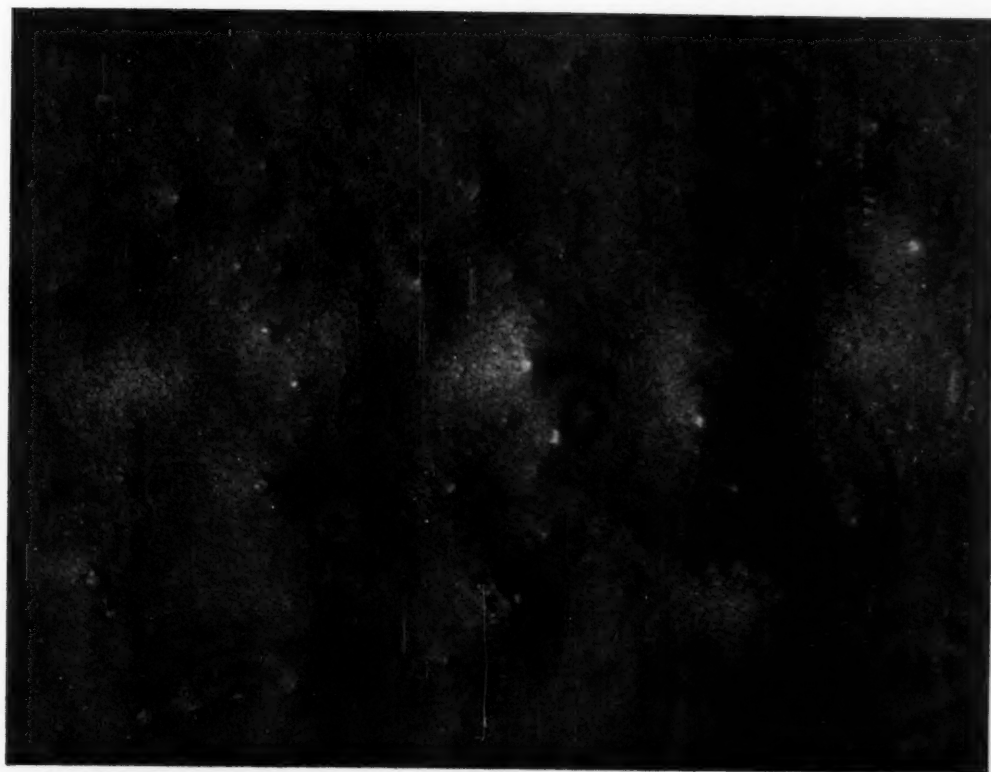


ACUTE CRAW-CRAW.





SMALL-POX.



VARICELLA.





# A PRELIMINARY NOTE ON THE PREVALENCE OF MOSQUITOES IN CAIRO AND ITS ENVIRONS

BY

F. C. WILLCOCKS,

ENTOMOLOGIST TO THE KHEDEVIAL AGRICULTURAL SOCIETY

*(Received for publication 15th February, 1910.)*

The Nile Floods of 1908 and 1909 were exceptionally high as compared with those of the previous three years. In 1908 the maximum level reached at Rhoda Bridge was 19.82 metres; this year (1909) the level was slightly less, viz., 19.69 metres. The average maximum heights for the years ending 1905-1907 was about 18.20 metres.

One of the results of such high Niles during the past two seasons has been to flood, by infiltration through the soil, the low-lying parts of Cairo and its environs. In many of these collections of infiltration water, mosquitoes breed in large numbers; the species varying to some extent with the kind of breeding-place. Fortunately, in the majority of cases the infiltration breeding-places are but temporary, persisting from two to three months only.

The breeding-places of this nature in Cairo and its environs are as follows:—

## I. CAIRO.

- A. Flooded building-sites — { excavated.  
unexcavated.
- B. Flooded basements of buildings (cellars)\*
- C. Flooded gardens.
- D. Disused sakia pits (water-wheel pits) in gardens.

## II. ENVIRONS OF CAIRO.

- E. Borrow-pits alongside roadway and railway embankments.
- F. Flooded garden and agricultural land.
- G. Sakia pits—not in use.

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\*Observation by Dr. E. H. Ross, Public Health Department.

Disused 'sakia' or water-wheel pits are permanent breeding-places, but when the water level in them is raised to near the surface of the land by infiltration from the Nile, it is possible that the species of mosquitoes breeding in them may differ, since the water will be more exposed to light.

In addition to the breeding places mentioned above, there are the cesspits, which supply Cairo with an unfailing supply of *Culex fatigans*, Wied., throughout the year, their numbers varying with the season (temperature). It is probable, however, that many old or little-used cesspits and wells, which at other times of the year are dry, become converted into breeding-places for Culicids during the flood.

In the case of gardens, agricultural land, and borrow-pits, flooded by infiltration from the river, one finds almost invariably that the surface of the water is overgrown with grass, water-plants and algae (*Spirogyra*, sp., *Hydrodictyon*, sp.). These places then form admirable breeding-sites for Anopheline as well as Culicine larvae. By about the middle of October, 1908, and again in 1909, collections of water of this nature were swarming with Culicine larvae and to a lesser degree with the larvae of *Cellia pharoensis*, Theob.

Such was the state of affairs on the Island of Ghezireh in October and November, 1908-1909. Ghezireh is situated opposite Cairo, and is connected with both banks of the river by bridges. On the island there is a large resident European population with their native and European servants; there are, in addition, a considerable number of native and European workmen, who go to and from their daily work. Again there is what may be described as a floating riverside population (chiefly natives) living in house-boats ('dahabiehs') moored against the banks, and in the ordinary Nile-boats engaged in the transport of merchandise.

As many parts of the island are low-lying they suffered considerably from flooding by infiltration from the river. *Culex*, spp. bred in countless numbers, and *Cellia pharoensis* in 'thousands.' *Grabhamia willcocksii*, Theob., was also very numerous. The females of this last species are vicious blood-suckers as well as plant feeders, but they do not appear to enter houses in any numbers.

Although *Cellia pharoensis*—a supposed malaria carrier—was very abundant in the autumn of 1908 in Ghezireh, yet, so far as the

writer is aware, there were no cases of malaria reported amongst the inhabitants, although the latter are derived from many parts of Egypt, and include also Europeans from India, the Southern Sudan, etc.

One might reasonably expect, therefore, that some cases of malaria would occur among such a population, and that in the presence of abundance of supposed carriers (*C. pharoensis*) an outbreak of malaria would occur, but this was not so.\*

One result of the collecting work carried out during the past two autumn seasons (1908-1909), has shown *Cellia pharoensis* to be an extremely common Anopheline in the environs of Cairo (it is believed to be the common Anopheline of Egypt). The apparent rarity, however, of malaria in this country, according to medical men, has raised doubts in the writer's mind as to the exact rôle played by *Cellia pharoensis* as a malaria carrier. Is this species a bad carrier, or a carrier at all?; or is the malaria carrier of Egypt some other much less common species of Anopheline which has so far been overlooked? Other Anophelines are also found in Egypt.

With regard to the question of the apparent rarity of malaria in the environs of Cairo one fact must not be lost sight of, namely, that the mosquito invasion takes place late in the year, and lasts a short time only—two or three months—and, moreover, does not, so far as is known, occur every year, but would appear to be dependent on the height of the Nile flood. It is possible that this has some bearing on the problem.

The habits of *Cellia pharoensis* are of interest. This species may be classed as a 'domestic mosquito' both in the larval and adult stages, but at present it is not known how far away from human habitations *Cellia* will breed. The adults enter houses in order to obtain blood. In the open the females appear to bite most viciously at sunset; their bite being rather painful compared with that of the common Culicine, *Culex fatigans*.

The aerial dances performed by male mosquitoes at sunset are perhaps worthy of note. The males of *Culex*, spp. dance in columnar form, well in the open, or in some cases, near or above bushes, and from fifteen to twenty feet from the ground. Thousands of individuals may be present in one of these dances, all in extremely rapid movements of limited range. They produce a very audible hum. The males of *Cellia pharoensis* dance as a rule in the open, but much

\*In November, 1909, however, one solitary case of malaria occurred in a cowman employed in the grounds of the Khedivial Agricultural Society.



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nearer to the ground than *Culex*, nor do they collect together in such large numbers. Their flight is also much slower. The aerial dances of the males of *Grabhamia willcocksii* are again different. They appear almost invariably to take place close to bushes, under trees or sheds; the males fly backwards and forwards with a slow and easy flight, about three feet from the ground, columns not being formed. One rather striking fact concerning these aerial dances is the comparatively small numbers of females which join them in order to pair with the males.

The mosquito conditions on the Gizeh side (west bank) of the Nile were the same as those which prevailed on Ghezireh both in 1908 and 1909. *Cellia pharoensis*, *Grabhamia willcocksii* and *Culex*, spp. were found in numbers, breeding in borrow-pits, flooded building-land, gardens and agricultural land. Many of these breeding places were located close to native villages, or to houses occupied by both Europeans and natives.

With regard to the City of Cairo, which is situated on the east bank of the river, one expected to find Culicids breeding in large numbers, but it was somewhat of a surprise, to find *C. pharoensis* breeding in the heart of a thickly populated native quarter of the city. At the end of November, 1908, a number of larvae were taken in a disused pit (water-level about 3 feet from the surface, and well lighted) in a garden in the Boulac district of Cairo. In November of this year (1909), this species was again found in the same garden, not in the sakia pit, but in a small pool of infiltration water.

On two occasions the writer has caught adult *Cellia pharoensis* in the Turf Club, Cairo. This building is situated too far away to be invaded by *Anophelines* from any of their known breeding places; the inference is therefore, that *Cellia* breed in other parts of Cairo.

In November of this year the writer obtained several records of the occurrence of *Cellia* (adults) in Cairo on the authority of Dr. H. E. Ross and others, and recently a new breeding-place was found in the Chubra quarter of Cairo.

Early in December, 1908, a new Anopheline was discovered in Cairo. This mosquito belongs to the genus *Pyretophorus*, and thus falls under suspicion as a possible malaria carrier. In a subsequent paper dealing fully with the mosquitoes found so far in Cairo and district it is proposed to describe this species under the name of *Pyretophorus cleopatrae*.

Breeding-places of this new Anopheline were found in the Gamrah and Chubra quarters of Cairo; also at Demadache, and at Heluan, about fifteen miles from Cairo. The waters in which the *Pyretophorus* larvae live is brackish, the dissolved sodium chloride varying from 0.366 per cent. to 2.6 per cent., and as a rule few or no grasses or water-plants have been present.

In the same pools the larvae of a new Culicid (n.sp. and ? nov. gen.) have also always been found, but neither of these species has—except on one doubtful occasion—ever been found associated with *Cellia pharoensis*, nor have they yet been found on the west bank of the river.

Larvae of *C. pharoensis*, both young and well-grown, placed in water containing 1.78 per cent. common salt, taken from a breeding-place of the *Pyretophorus* larvae, died in less than twenty-four hours. In water containing 1 per cent. common salt, also from a *Pyretophorus* breeding-place, *Cellia* larvae lived from two to three days, but during this period until death took place they were very sluggish in their movements and appeared to feed very little or not at all, though in each case food was provided for them.

The more distant environs of Cairo visited were Marg and Ezbet el Nakhl to the North, and Toura and Heluan to the South. In both the former places *Cellia pharoensis* was common, also *Culex*, spp. and *Grabhamia willcocksii*.

At Toura, *Cellia pharoensis* breeds in borrow-pits situated in the desert, some close to the railway, others further away. Except in one case neither grass nor reeds grew in these pits, but the surface of the water was overgrown with algae, which provided protection as well as food for the larvae. It is of interest to note that a few years ago, there was an outbreak of malaria amongst the convicts at Toura prison. The prison buildings are within a short distance of the borrow-pits mentioned.

At Heluan, in pools in marshy ground lying immediately to the South-west of the town the larvae of the new *Pyretophorus* were found in large numbers. The water in these pools contained from 2.56 per cent. to 3.25 per cent. of common salt. In a pool to the North of the town the *Pyretophorus* larvae were found associated with the larvae of *Grabhamia willcocksii*, Theobald, and *Theobaldinella spathipalpis*, Rondani. The water in this pool contained 1.6 per



cent. common salt. Both the pools to the North and South of the town appear to be fed by springs.

*Cellia pharoensis* was not found at Heluan.

In previous years, cases of malaria are said to have occurred in Heluan.

During the collecting work carried out in 1908 and 1909, a number of natural enemies of larvae were observed. The most important of these are aquatic bugs (Hemiptera), commonly known as 'back-swimmers' (so named from their habit of swimming on their backs), belonging to the genus *Notonecta*. This genus has a wide geographical distribution, and its members are recorded by observers in other parts of the world as natural checks on the increase of Culicidae. In 1908 a large area of the Ghezireh grounds of the Khedevial Agricultural Society, flooded by infiltration water, was largely freed from larvae of *Culex* spp. and *Cellia pharoensis* through the agency of these aquatic bugs, and the same has been observed in other localities.

These bugs appear to increase rapidly and to become quickly distributed in a district. The adults leave the water with ease and take flight, but if in the course of their flight they hit an obstacle and fall, they appear to have very great difficulty in again taking wing or even to be unable to do so. They leave the water with the dorsal surface upwards making a slight whirring noise.

Another but rather rare member of the same family of water-bugs (*Notonectidae*) has also been found to prey upon mosquito larvae, and although these insects measure but 2 mm. in length they will attack and destroy almost full-grown mosquito larvae.

Other natural enemies noted were water-boatmen (*Corixidae*), aquatic beetles and their larvae, and the larvae and nymphs of dragon-flies (*Libellulidae*).

The 'back-swimmers' of the genus *Notonecta* must undoubtedly be placed at the head of the list. It is unfortunate that these useful insects do not as a rule inhabit quite shallow waters, and also that their mosquito destroying operations are hindered to some extent, by growths of grass water-plants and algae, which protect their prey.

With regard to the enemies of adult mosquitoes, bats appear to catch them at sunset, dragon-flies probably destroy some also, but in any case it is probable that the greater proportion of mosquitoes destroyed in this way are males.



In conclusion the writer would urge the necessity of a thorough investigation of the question of malaria in Egypt, and especially a study of *Anophelines* likely to act as carriers of the disease. In Ismailia, malaria was at one time rife; it occurred at Port Said\*, and it is said to occur in the Western Oases, and to have a decided injurious effect on the inhabitants. If malaria exists or has existed in such widely separated localities—though they may not be entirely representative in some respects of the country as a whole—why does it not occur more commonly in other parts of Egypt? The country in the North of the Delta is full of canals—which in a great many cases are full of grasses and other plants—stagnant drains, marshes, and last, but not least, rice fields; in other words a country well suited to breeding *Anophelines*, which are known to occur there at certain times of the year and in certain localities in large numbers. Yet one is informed that malaria is rare in Egypt, and so far no satisfactory reason has been given for this apparently anomalous condition. It is because of the lack of exact information on this problem in Egypt that the writer ventures to put forward a plea for further work on the question. If investigation proves that malaria is rare, as is generally stated, so much the better for Egypt. Moreover the utility of this work would not end here, as it is probable that the explanation of the immunity of Egypt, if it is a fact, would throw light on many imperfectly understood problems of malarial distribution elsewhere.

The writer would here tender his sincere thanks to the many persons who have aided him in the work which forms the subject of this paper.

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\* Ross, E. H. The prevention of Fever on the Suez Canal. Cairo, 1909, p. 14.

The following is a list of the names of the persons who have been appointed to the various offices of the County of ...

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## THE EFFECT OF MOSQUITO LARVAE UPON DRINKING WATER

BY

RUBERT BOYCE, F.R.S., AND F. C. LEWIS

*(Received for publication February 26, 1910.)*

In all yellow fever countries, it is well known that the presence of *Stegomyia* larvae in any collection of water is a source of danger, so much so, that in very many British Colonies penalties are now enforced against stagnant water, and the presence of mosquito larvae is deemed evidence of this.

If the water cannot be thrown away, then it must be securely protected by netting from Mosquitos, or it must be oiled or stocked with larvae-destroying fish.

It so happens that the larvae of the *Stegomyia* are most commonly found in clean water, the *Stegomyia calopus* selecting clean water to deposit her eggs in. Clean water is of course the water usually found in and around all houses, being required for domestic use, for drinking and cooking purposes, baths, flowers and for any domestic pets or animals kept on the premises. It, therefore, is not to be wondered at that in the yellow fever zone the presence of larvae is taken as evidence that the water is clean. But their presence is only evidence that the clean water was in all probability the only available water near to or in the house found by the *Stegomyia* in which to deposit her eggs. From the fact that *Stegomyia* larvae were usually met with in clean water, they became in time to be regarded in a beneficial light—to possess in fact a clarifying influence upon water. They were regarded as scavengers, taking into their interiors any bacteria or other minute organisms which might be present. The normal food of mosquito-larvae consists of minute forms of vegetable matter, and therefore they no doubt do consume bacteria. Hence it was argued that their presence in water barrels supplied from the roofs of houses would be distinctly useful, for it was urged that they might consume pathogenic bacteria derived from the dust of the roofs or from the

droppings of birds of the carrion-feeding kind. This belief considerably influenced some authorities against rigorously protecting their water supplies from mosquitos, and it was therefore with the object of testing whether there was any foundation for this belief and with the further object of studying the effect of minute forms of animal life on drinking water—such forms, for example, as are found in large numbers in the filter beds and reservoirs of the water supplies of cities—that I undertook with my Assistant, Mr. Frederick C. Lewis, the following series of simple experiments:—

The experiments consisted in placing larvae of *Culex* spp. and of *Theobaldia annulata* in a flask of non-sterilised drinking water, and comparing from day to day the number of bacteria present in the water with the number present in a control flask to which no larvae had been added. The two flasks which I call 'A' and 'B' were freely exposed to the air. The number of bacteria was estimated by plating 1 c.c. of the water in gelatine and incubating at 21° C. for 72 hours.

The results were as follows:—

#### SERIES I

Date	FLASK 'A,' without larvae	FLASK 'B,' with larvae	Date
	Bacteria per 1 c.c.	Bacteria per 1 c.c.	
4.12.09	880	873	4.12.09
5.12.09	983	1,935	5.12.09
6.12.09	1,350	2,720	6.12.09
7.12.09	1,420	2,590	7.12.09
8.12.09	765	3,597	8.12.09
9.12.09	980	5,390	9.12.09
10.12.09	87	—	10.12.09
11.12.09	15	—	11.12.09
12.12.09	35	9,370	12.12.09

#### SERIES II

Date	FLASK 'A,' without larvae	FLASK 'B,' with larvae	Date
	Bacteria per c.c.	Bacteria per c.c.	
28.1.10	25	1,250	29.1.10
30.1.10	109	8,600	30.1.10
31.1.10	599	51,200	31.1.10
1.2.10	3,090	67,000	1.2.10
		137,000	3.2.10



## SERIES III

Date	FLASK 'A,' without larvae	FLASK 'B,' with larvae	Date
	Bacteria per c.c.	Bacteria per c.c.	
5.2.10	27	56	5.2.10
7.2.10	193	149,000	7.2.10
8.2.10	1,760	168,000	8.2.10
9.2.10	3,920	...	9.2.10

## SERIES IV

Date	FLASK 'A,' water to which typhoid bacilli were added, but no larvae	FLASK 'B,' same water with larvae added	Date
	No. of <i>B. typhosus</i> present per c.c.	No. of <i>B. typhosus</i> present per c.c.	
5.12.09	12	13	5.12.09
6.12.09	16	Large increase of <i>B. typhosus</i> and other bacteria	6.12.09
7.12.09	6	" "	7.12.09
8.12.09	3	50	8.12.09
9.12.09	—	7	9.12.09
11.12.09	—	6	11.12.09
12.12.09	—	0	12.12.09

## SERIES V.—To ascertain the effect on drinking water of the Water Cyclops.

Date	FLASK 'A,' without Cyclops	FLASK 'B,' with Cyclops	Date
	Bacteria per c.c.	Bacteria per c.c.	
28.1.10	25	1,060	28.1.10
30.1.10	109	1,460	29.1.10
31.1.10	599	14,200	30.1.10
1.2.10	3,090	48,600	31.1.10
		60,000	1.2.10
		173,000	3.2.10

## Result:

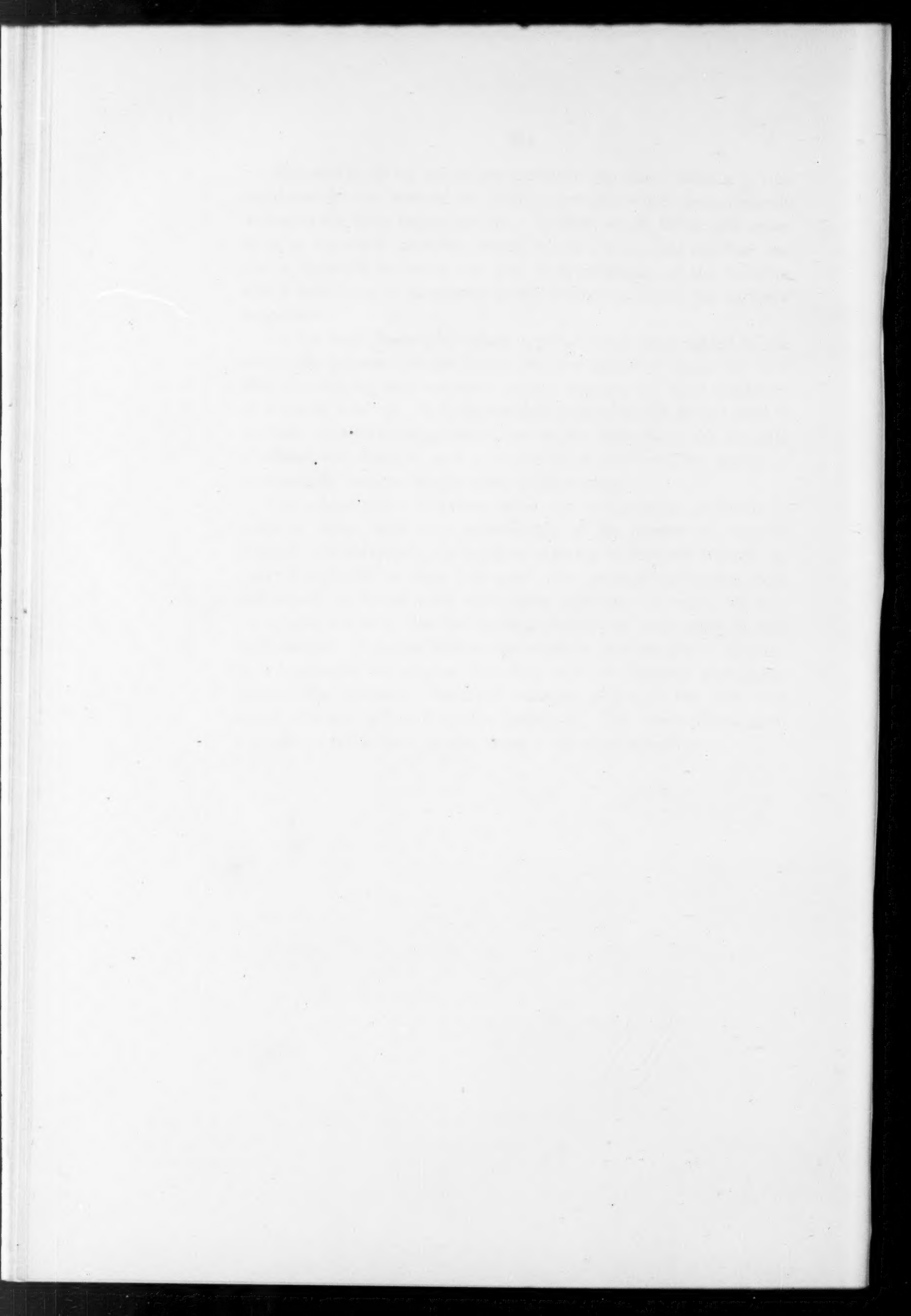
From these experiments, it will be seen that in clean drinking water drawn from the tap and exposed to the air, there is a slight multiplication of the number of bacteria for a few days, and that then the bacteria rapidly decrease, in all probability owing to the want of food material.

If, however, living larvae are placed in the water there is a very rapid rise in the number of bacteria per c.c., which is enormously increased if a larva happens to die. In other words, larvae add something to the water, probably mucus, which acts as food material, and which therefore increases the rate of development of the bacteria, and a dead larva in decomposing still further increases the bacterial proportion.

In the case (Series IV) where typhoid bacilli were added to the water, the presence of the larvae did not appear to have the least effect in reducing their numbers; on the contrary, the total number of all bacteria went up. It is known that typhoid bacilli do not tend to multiply in clean drinking water, but, on the other hand, the presence of albuminous material such as would be derived from the bodies of larvae might tend to favour their multiplication.

The experiments, therefore, show that the presence of larvae in drinking water adds very considerably to the number of bacteria present. In the tropics, the number of larvae to be found in drinking-water receptacles is often very great, and amongst the larvae, there will usually be found some which have perished; therefore, we may reasonably conclude that the bacterial content of such water is very high indeed. If larvae lead to the increase of saprophytic bacteria, it is reasonable to suppose that they will not diminish pathogenic forms. The evidence, therefore, strongly points to the fact that larvae in water will still further pollute it. The observations upon *Cyclops*, as far as they go also point in the same direction.











Volume III

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No 5

ANNALS  
OF  
TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY

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By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fifth number of the third volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.



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